Abstracts

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Title:
Inhibition of Bacterial Biofilms by Antimicrobial Peptides: Take a Glance at the Parameters Affecting the Efficacy of Surface-Tethered Antimicrobial Peptides

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Abstract:
Due to intrinsic resistance to conventional antibiotic therapy, bacterial biofilms represent serious threat to human health. Thus, development of efficient biocidal materials which inhibit the colonization of pathogens at surfaces is an approach to combat surface-associated infections. One strategy is the covalent attachment of antimicrobial peptides (AMPs) to such biomedical surfaces. Here, the impacts of AMPs mode of actions as well as physical characteristics of surfaces upon antimicrobial activities of surface-covered peptides are discussed. PEGylated resin beads with same distribution of size, but various PEG spacers and loading capacity were used as model solid surfaces. KLAL and MK5E with carpet-like mode of action, channel-forming melittin, and buforin 2 with intracellular targets were AMPs in this study. Peptides were tethered at C and N terminus and via different side chain positions to the solid surface. The influence of resin bead parameters; e.g., spacer length and density, and peptide mode of actions were assayed upon the antimicrobial and bilayer permeabilizing activities of tethered peptides. Our results showed: - Reduction of spacer length between the solid surface and active sequences leads to a decrease in the kinetics of bactericidal growth independent of amount of loaded peptide on solid surface. - Membrane active AMPs are suitable for the generation of antimicrobial surfaces. - Depending upon the peptide mode of actions, the coupling position affects the peptide activity; e.g., the activities of tethered KLAL is independent of coupling position, but N terminally tethered melittin is less active as compared with the C terminally tethered peptide.

Keywords: Bacterial biofilms, Antimicrobial peptides, Peptide tethering, Lipid bilayer permeabilization
Title: Bradykinin Effect on IP3 Release in HEK293T Cells by Luminescent Biosensor Monitoring

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Abstract: Bradykinin, a vasoactive peptide (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), is a biologically active hormone, which stimulates many cellular functions such as phosphatidylinositol turnover, through a mechanism requiring the influx of extracellular calcium and activation of phospholipases followed by hydrolysis of phosphatidyl inositol 4,5-bisphosphate (PIP2) and formation of IP3. To check out the ability of the endogenously released IP3 by bradykinin in HEK293T Cells, the selected cell line transfected with IP3-biosensor and monitored by the luciferase activity with in vitro cell-based assay. The intact M bradykininµtransfected cells were investigated after treatment with 1 over time (for 0, 15, 30, 60, 120 and 300 sec); then cells harvested and assayed to monitor the luminescence activity. The our results shown that significant level of the photon emission was observed as result of the ligand interaction and subsequent luciferase activity that confirmed release of IP3 into target cells. Compared with untreated transfected cells, bradykinin induced approximately 8.0-fold increase in the luciferase activity within 30 sec after its addition, thereafter the signal declined back to base-line level gradually. In order to obtain response curve to bradykinin, its various concentrations were tested on IP3 release and monitored by luciferase assay. The transfected cells were harvested after 48h and stimulated with increasing bradykinin M for 30 sec, next the luciferaseµconcentration from 1 nM to 10 activity was measured. No change in signal was observed at concentrations lower than 10 nM. The maximum response in the presence of M bradykinin was 8.0-fold stronger than that in the absence of µ1 bradykinin. No further increase in luciferase activity was observed upon addition of higher concentrations of bradykinin.

Keywords: Keywords: Bradykinin; IP3; biosensor; HEK293T Cells.
Title:
Inhibition of Quorum-Sensing Controlled Virulence Expression in Enteropathogenic Escherichia coli by Berberis vulgaris L. var Asperma and Blockade of Intimin-Mediated Enterocyte Attachment In Vitro

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Abstract:
Introduction: Enteropathogenic Escherichia coli (EPEC) is a significant cause of diarrheal disease worldwide. This pathogen intimately attaches on the surface of human enterocytes secondary to expressed virulence factors believed to be quorum-sensing (QS) mediated. The QS inhibition (QSI) property of several phytochemicals from ethanolic extracts of Berberis vulgaris L. var. Asperma (zereshk) against EPEC pathogenesis and virulence was investigated.

Method: Transcription profiles of virulence factors, eaeA (intimin), escC (type III secretion biogenesis), and tir (translocated intimin receptor) were respectively quantified by real-time quantitative PCR post-exposure to zereshk ethanolic extract. Moreover, co-treatment of EPEC planktonic cultures with ethanol extract seeded in human HT-29 cells was performed in vitro to determine bacterial attachment and colonization. HT-29 cells and EPEC were correspondingly stained with fluorescent probes and visualized microscopically. In silico molecular interaction of major phytochemicals, previously identified by liquid chromatography-gas chromatography (LC-MS), on the intimin binding site was assessed via AutoDock. Results: A significant reduction in the expression of eaeA, escC, and tir (p<0.05) was observed from as low as 20% of the extract. Fluorescence photomicrography revealed inhibition of localized adherence opposed to untreated controls. Additionally, chlorogenate, columbamine, rutin, isocorydine and rutin showed high dock scores according to their molecular interaction with intimin. Conclusions: Results infer the QSI potential of B. vulgaris against EPEC as evidenced by non-adherence on HT-29. These findings suggest prevention of infection and colonization of EPEC as shown by the efficient blockade of intimin by the bioactive compounds identified.

Keywords: Berberis vulgaris L., enteropathogenic E. coli, quorum sensing inhibition, intimin
Title:
Isolation of human SCFV antibody against EGFR L2 domain by screening of human SCFV phage library

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Abstract:
Introduction: Epidermal growth factor receptor (EGFR) is one of the key molecules in cell growth and multiplication and plays an important role in some malignant processes. L2 domain of extracellular part of this receptor involved in ligand binding and its inhibition can prevent activation of related signaling pathways. The aim of the present study was isolation of a human single chain antibody against L2 domain of EGFR by phage display technique. Materials & methods: A large phagemid library of human scFv was used for isolation of L2-specific scFv clones. The library was screened by biopanning technique for 5 cycles using recombinant L2 protein. The reactive clones were further confirmed by ELISA and western blotting analysis. Results: Screening by biopanning resulted in isolation of several reactive clones among them 1 clone with higher affinity was selected for further studies. Analysis by ELISA and western blotting revealed that the selected clone is reacted with recombinant L2 and EGFR expressing A431 cells. Conclusions: The results of this study showed that the selected scFv is specifically reacted with target antigen and can be used as a potentially used for development of human anticancer drugs.

Keywords: Single chain antibody, EGFR, Phage display
Title:
Extraction, purification and kinetics study of Butyrylcholinesterase from Equine plasma

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Abstract:
Introduction: Butyrylcholinesterase (BChE) is interest of particular military, medical, agriculture etc. It hydrolyzes a wide range of toxic compounds such as cocaine, succinylcholine and carbamate pesticides. Method: BChE was purified from Equine plasma using polyethylene glycol (PEG) differential precipitation and ion exchange chromatographic procedures. Kinetic parameters of purified enzyme were obtained in 37 degree of centigrade. Results: The final purified BChE showed a single electrophoresis band (85 kDa) on SDS-PAGE. Purification was resulted in a homogeneous enzyme preparation with a 5575-fold purification. Kinetics parameters of the enzyme were obtained, Km = 667 µM and Vmax = 3.33 E-7 µM/s. Specific activity of BChE was estimated as 408 U/mg with a reasonable final yield (15%). Conclusions: The procedure that we used for purification of Equine BChE is inexpensive, simple and comparable to procainamide affinity column chromatography.

Keywords: Butyrylcholinesterase (BChE), Equine plasma, purification, Enzyme Kinetic.
Title: Catecholic Complexation of Vanadium Stabilizes highly Sensitive +3 oxidation State A Kinetic and Mechanistic Approach

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Abstract:
Introduction: Simple aromatic compounds bearing phenolic and carboxylic groups present in biological systems serve as versatile ligands to chelate toxic and nutrient metal ions. Many of these compounds that found in natural environment as metabolic and degradation processes may act as non-specific ligands towards metal ions in terrestrial and aquatic system. With this class of ligands, very efficient chelating ability is achieved through several potential donor sets e.g. of salicylic and catecholic type. Catecholate, the oxygen donor ligands, have high affinity towards transition metal ions. They can stabilize the highly sensitive (+3) oxidation state of vanadium even in presence of oxygen. Method: A number of catecholic ligands were tried, out of which three (pyrocatechol,gallic acid and gallic acid methyl ester) were chosen for the detailed study. The substituted group on a catecholic ring helps to understand the binding sites of the ligand. To evaluate the stability constants of these species, potentiometric and spectrophotometric methods were implied. Overall stability constants with least s* fit value were evaluated by computer program BEST. Results: Catechols form highly colored stable complexes with vanadium(III) in 1:1, 1:2 and 1:3 M:L molar ratio. Log K value for the attachment of first ligand did not vary substantially with the difference in the ring substituents on the catechols. However stability constants for second and third chelate rings are distinctly affected by substitution on the catechol rings which is attributed to intraligand hydrogen bonding. Gallic acid complex of V(III) showed highest values of stability constants, $b_1$ is $10^{14}$, $b_2$ is $10^{17}$ and $b_3$ is $10^{20}$. The b calculated from the spectrophotometric data is also comparable. Kinetics and rates of formation were determined for each complex system and mechanism of the reactions was proposed. References: 1. K. Ali, N. Fatima, S.A. Kazmi and Z.T. Maqsood. Complexation of Vanadium(IV) with Hydroxamate Chelators and their Stability Relation with pH. J. Iran Chem. Soc., 1(1), 65-70, 2004. www.ics-ir.org/jics. 2. N. Fatima, S.Z.A. Zaidi, S. Nisar and M. Qadri. pH Effect on Stoichiometry and Stability of Ferrous Complexes of (-)-3-(3,4-dihydroxyphenyl)-L-alanine. Pak. J. Chem., 3(1), 1-6, 2013. www.pjchm.com. 3. N. Fatima, Complexation, Stability and Stoichiometry of Iron(III) with Salbutamol (Active ingredient of Asthma drug Ventolin®). Pak. J. Chem., 2(2), 91-98, 2012. www.pjchm.com. 4. N. Fatima and Z.T. Maqsood. Study of Formation Constants of Vanadium(III)-Catecholate Complexes. J.Saudi Chem. Soc. 9(3), 519-528, 2005. 5. K. Ali, Z.T. Maqsood and N. Fatima. Comparative study of Thermodynamic Parameters of Oxo-oxovanadium(IV) and (V) Acetohydroxamate Complexes. Scientia Iranica. 12(3), 311-317, 2005

Keywords: Catechols, spectrophotometry, potentiometry, stability constants, kinetics.
Title:
Effect of Chemical Functionalization of Carbon Nanotubes on the Electrochemical Behavior of Choline Oxidase

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Abstract:
Introduction: This work presents a comparison between nano-composites containing carbon nanotubes (CNT) with different functional groups and same room temperature ionic liquid (RTIL) in order to investigate the effect of CNT functional groups on the electrochemical and electroanalytical response of choline oxidase (ChOX).

Method: CNTs were functionalized with carboxyl, amine or amide groups. Carboxylated CNTs (HOOC-CNTs) were obtained by acid treatment. Amine functionalization of CNTs was done chemically using ethylenediamine (EDA-NH$_2$-CNTs) or tetraethylenepentamine (TEP-NH$_2$-CNTs); while, amid functionalization of CNTs was done physically by ammonia plasma treatment (p-NH$_2$-CNTs). ChOX was absorbed on different CNT/RTIL modified electrodes and its electrochemistry and catalysis toward choline was investigated.

Results: The resulting data showed that the electrodes modified with p-NH$_2$-CNTs had higher apparent heterogeneous electron transfer rate constant ($k_u$) (2.74 s$^{-1}$), indicating more facile and lowest rates of electron transfer. While, electrodes modified with TEP-NH$_2$-CNTs showed the lowest detection limit of 5.81*10$^{-6}$ M for choline and the most sensitive electrocatalytic response (1.09*10$^3$ A M$^{-1}$ m$^{-2}$).

Conclusions: In conclusion, p-NH$_2$-CNTs were more convenient for electrokinetic study of the enzyme, where there is a need for facile and rapid electron transfer, while TEP-NH$_2$-CNTs were preferable for choline biosensing applications.

Keywords: Carbon nanotubes, Functionalization, Ionic liquid, Choline oxidase
Title:
Homocysteinylation of lens Crystallins: From Protein Aggregation to Possible Risk Factor in Development of Cataract Disorders.

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Abstract:
There are several evidences suggesting a relationship between hyperhomocysteinemia and various ocular disorders such as cataract, ectopic lenses, open-angle glaucoma, pseudoexfoliation glaucoma, central retinal vein occlusion, maculopathy, optic atrophy and diabetic retinopathy. Since the side chains of Lys residues are modified not only by non-enzymatic glycation, but also by homocysteinylation; the two modifications may have similar structural and functional consequences. In this study, different spectroscopic techniques, gel electrophoresis under reducing and non-reducing conditions, and western blot analysis were applied to evaluate role of homocysteinylation on structure and function of eye lens crystallins. Homocysteinylation of crystallin proteins causes significant structural alterations, leading to aggregation and fibrillation of these proteins. The chaperone activity of α-crystallin which is important for transparency and refractive power of eye lens was reduced after this modification. Also homocysteinylated α-crystallin demonstrates significant propensity for aggregation and precipitation in the test tube. The aggregation of homocysteinylated crystallins is of medical importance because it is believed that slow aggregation and precipitation of these proteins as result of various modifications which accumulate over years is the molecular basis of some types of cataract. Overall this study may suggest lens protein homocysteinylation as a possible risk factor in the development of the cataract disorders.

Keywords: Eye lens crystallins, Homocysteinylation, Fibrillation, Cataract disorders.
Title:
Gold nanoprobes: tools for imaging and therapeutic purposes

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Abstract:
Nano probes represent great promise for the diagnosis and treatment of human diseases. These probes with their unique optical properties have been employed for detecting a variety of pathogenic microorganism including bacteria, viruses and fungi. *Escherichia coli*, *P. syringae*, *Influenza* and *hepatitis* virus are among the pathogens detected by nanoprobes. They have also gained significance as novel pharmaceutical compounds to be used for imaging purposes and targeted delivery of therapeutic cargoes into cancer cells. This indicates the potential of nanoprobes for the diagnosis and cure of disseminated and aggressive tumors. Gold nanoprobes, 10-100 nm in diameter, are functionalized by conjugation with biofunctional groups such as thiol. Colorimetric methods for gold nanoprobe-based detection of biological targets (DNA, RNA, protein, aptamers and lipids) which are implicated in the emergence of diseases are rapid, easy and sensitive for clinical applications. The colorimetric detection is performed through color change resulting from aggregation of nanoprobes. Taken together, the special features of nanoprobes and their diverse range of applications highlights their importance as valuable diagnostic and therapeutic tools.

Keywords: Gold nanoprobes, imaging, diagnosis and treatment
Title:
Study on the structural and binding properties of different variants of bovine β-lactoglobulin

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Abstract:
Introduction: β-Lactoglobulin is a small globular protein of 162 amino acid residues with a molecular mass of 18.36 kDa. Its biological role is still unknown, and many studies have suggested a nutritional and a specific transporter role. The high affinity of β-Lg to retinol and other retinoids was reported. The results of interaction study of β-Lg with carotenoids such as β-carotene, β-cryptoxanthin and α-carotene, which display similar structure are reported. In the present work, the affinity of different variants of β-Lg to retinoids and carotenoids is compared and more informations provided about the binding site of these molecules on β-Lg.

Material: Interactions of these molecules to β-Lg were followed by the measurements of quenching of both β-Lg tryptophan and ligand fluorescence. The binding affinity constants were obtained for all ligands.

Results: The obtained results indicated that carotenoids are bound by β-Lg with high affinity. Measurement of retinol competition with carotenoids for binding suggests that the binding of these two ligands occurs at two different sites of β-Lg. The obtained results remain in agreement with the hypothesis of binding of retinol and palmitate at the same interior cavity.

Conclusion: The obtained results indicated that β-Lg variants A and B have almost the same interactions with retinoids and carotenoids. It has been showed that in spite of the high hydrophobicity of β-carotene, it has an important affinity to β-Lg in comparison with other studied molecules.

Keywords: β-Lactoglobulin, Binding affinity constant, Competition experiment, Retinoids, Fluorescence spectroscopy.
Title:
Improving the stability and antioxidant activity of curcumin upon interaction with native and acetylated forms of albumin, casein and β-Lactoglobulin: A comparative study with perspective of functional food application

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Abstract:
Introduction: Curcumin is a natural polyphenolic compound with anti-cancer, anti-inflammatory, and anti-oxidation properties. But, low water solubility and rapid hydrolytic/photonic degradation are two challenges that limit use of curcumin. In this study, the role of the native/modified forms of serum albumin, casein and β-Lactoglobulin, as food-grade biopolymers in the improvement of stability and antioxidant properties of curcumin were surveyed.

Method: Proteins were modified with acetic anhydride which offers neutral acetyl groups usually at lysine side chains. Hydrolytic and photonic degradation of curcumin and its interaction with native/modified proteins were studied using UV-vis and fluorescence spectroscopy, respectively. Also, antioxidant potency was measured by DPPH assay.

Results: Upon interaction of curcumin with the native proteins, its hydrolytic/photonic degradation was significantly suppressed in the order of serum albumin>casein>β-Lactoglobulin. Also, rate and extent of DPPH oxidation was greatly decreased in the presence of curcumin and protein-bound curcumin with the same manner. But, acetylation changed capability of proteins in the improvement of stability and antioxidant properties of curcumin in the order of β-Lactoglobulin>albumin>casein. Analyses of curcumin interaction with the proteins revealed that binding of curcumin to the hydrophobic core of proteins increases its efficacy more than surface hydrophilic regions.

Conclusions: These findings imply that proteins such as native albumin/casein and acetylated β-Lactoglobulin as a natural biopolymer from serum and milk can be a good matrix for increasing the functionality of curcumin in food.

Keywords: Curcumin, Antioxidant, Chemical stability, Functional food, Protein chemical modification.
Title:
DEVELOPMENT OF MICROENCAPSULATED NUTRACEUTICALS; MODERN TECHNOLOGY

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Abstract:
Nutraceuticals are extracted from food source, dietary substance or traditional herbs and delivered in the pharmaceutical dosage forms such as pills, tablets, capsules, liquid orals, or other dermal preparations. The word nutraceutical has been invented to define natural, non-toxic dietary supplement design to improve health nutrition. These are developed according to the pharmaceutical principles and evaluated in process control to ensure the reproducibility and therapeutic efficacy of the active ingredient. Most of the nutraceuticals companies especially working in high-protein diets provide scientific background to support their products and ensure health claims. There is a dire need to develop various innovative technologies that allow pharmaceutical companies to understand the target of the active compound in the actual biological system. Some modernized nutraceutical companies use recent analytical tools to evaluate metabolomics, biomedical pathways to help the clients. They can sort out early profiling of safety issues, off-target effects, discover new drug targets, submit required regulations, and to take the correct decisions. Concept of microencapsulation is one of the latest technologies used for nutraceuticals that may reduce several issues regarding product. Using appropriate encapsulation may increase the range of products by providing low hygroscopicity, optimum spreadability and proper release of active ingredients. There are also some benefits of microgranulated form like free-flowing, disperses and dissolves quickly, good tableting properties and can therefore be considered reliable. Encapsulating material will not impart any taste or color to the formulation therefore, it may use in various other applications.

Keywords: Functional and Nutraceutical Foods, Drug discovery and Nutraceuticals and Health Concerns
Title: Applications of bioinformatic and computational tools to identify deleterious functional SNPs

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Abstract:

Introduction: Single nucleotide polymorphisms (SNPs) represent the most abundant division of genetic variations in the coding region and noncoding including untranslated regions (UTR) of the human genome. Detection of function of nonsynonymous SNPs (nsSNPs) that causes to change the amino acid residues in susceptibility to general diseases is a major goal in human genetics. However, the method to recognize functional SNPs from neutral SNPs of the human genome is challenging by experimental protocols. Computational detection of deleterious SNPs and their effect on sequence and structural level is utmost important. Herein, the deleterious nsSNPs that can alter the expression and function in the cancer related genes such as TP53 have been explored based on computational bioinformatics algorithm. The TP53 tumor protein is essential for regulating cell division that mutated in more than 50% of human cancers.

Method: In silico methods applied to examine the genetic variations including nonsynonymous coding SNPs, UTR SNPs and introns that can alter the expression and function of the cancer related gene. For this aim, different computational algorithm like a sequence homology-based Sorting Intolerant from Tolerant (SIFT) server, structure-based method via PolyPhen server, FASTSNP, Pupa Suite software and Yet Another Scientific Artificial Reality Application software (YASARA) were used. Molecular modeling studies applied to compare mutant proteins with the native protein to evaluate the stability and functional activity of TP53.

Results: The possible mutations and proposed modeled structure for the mutant proteins explored. The sequence homology-based SIFT program prediction indicated the possibility to exist 209 SNPs TP53 gene. Protein sequences of nonsynonymous SNPs (nsSNPs) were explored by SIFT program and 14 nsSNPs recognized to be deleterious. Structure-based method of PolyPhen confirmed the data from SIFT program about structural damaging effect of nsSNPs. Molecular modeling studies to compare separately mutant proteins at the corresponding positions with the native protein showed lower stability of deleterious mutant-type structures. Data indicate the deleterious nsSNPs caused to change the protein structural that result in decrease interaction of TP53 protein with DNA. The structural changes via nsSNPs that affect protein-DNA interactions possibly are the main reasons for incapability of mutant TP53 to suppress the occurrence of different cancers.

Conclusion: Our results obtained through computational algorithms and molecular modeling imply that the nsSNPs play a critical role in cancer association studies aiming to improve the effectiveness of the cancer treatments. Our results support and providing a guide to study with in vivo experimental procedures.

Keywords: SIFT, PolyPhen, TP53 gene, Modeling
Title:
The nanoparticle based optical and electrochemical immunosensor for detection of hepatitis B surface antigen

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Abstract:
The high affinity of antigen-antibody interaction is the basis for precise detection and diagnosis of antigens by means of immunosensor. In the present study, immunoassay was incorporated with two strong physical methods: chemiluminescence and electrochemistry, to develop the immunosensor for detection of hepatitis-B virus at low concentration. In the first method hepatitis B surface antigen (HBs-Ag) was detected by a chemiluminescence immunosensor. In a sandwich type immunoassay method, the primary antibody (anti-hepatitis B surface antigen, anti-HBs-Ag) was immobilized in polystyrene wells and the secondary antibody was conjugated to luminol coated gold nanoparticles (GNPs) as label. Then, HBs-Ag was conjugated between primary and secondary antibodies. The immunosensor responded toward HBs-Ag in a wide linear range of 0.125 to 30 ng/ml. The proposed method has successfully applied to determine the HBs-Ag in patient sera with a detection limit of 14 pg/ml (signal/noise=3). In the electrochemical method, the biotinylated hepatitis B surface antibody was immobilized on streptavidin-magnetic nanoparticles and used for targeting the HBsAg. In the presence of horseradish peroxidase conjugated secondary antibody (HRP-HBsAb), a sandwich-type immunoassay format was formed. O-aminophenol and hydrogen peroxide as substrates for HRP were used to produce 3-aminophenoxazone (3-APZ). The electroactive enzymatic production of 3-APZ was transferred into an electrochemical cell and monitored by voltammetry. Under optimal conditions, the cathodic current response of 3-APZ which was proportional to HBsAg concentration was measured by a glassy carbon electrode. The immunosensor response was linear towards HBsAg in the concentration range from 0.001 to 0.015 ng/ml with a detection limit of 0.9 pg/ml (signal/noise=3). Both proposed methods were successfully applied to determine the HBsAg in patient sera.

Keywords: Hepatitis B surface antigen, Luminol, Gold nanoparticles, Chemiluminescence, Immunosensor; Magnetic nanoparticles; Aminophenole; Aminophenoxazone
Title:
3D QSAR study for a series of TNF-alpha converting enzyme inhibitors

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Abstract:
Introduction: Tumor necrosis factor-alpha (TNF-α) is a multifunctional cytokine playing an important role in cell proliferation, differentiation, inflammation, death and immune regulation. Increased level of TNF-α is involved in the variety of inflammatory diseases, such as rheumatoid arthritis and Crohn's disease. TNF-α converting enzyme (TACE) is responsible to release TNF-α from membrane anchored preformed into its soluble and active form. Therefore, inhibiting this enzyme could provide a new area for designing novel TACE inhibitors effective in treatment of inflammatory diseases. Methods: Three dimensional Quantitative structure-activity relationship (3D-QSAR) methods are used to predict the pharmaceutically relevant properties of drug candidates whenever it is applicable. In this study a set of TACE inhibitors was docked into the active site of the enzyme using GOLD program. Development of 3D-QSAR models was performed by applying partial least square (PLS) and principle component analysis (PCA) methods implemented in GRID/GOLPE and Pentacle softwares. The reliability and validity of the proposed models were evaluated using leave-one-out (LOO) and leave-group-out (LGO) cross validation methods. Results and Conclusions: The results showed that the generated models were able to predict accurately (r²=0.95, q²=0.7) the inhibitory activity of the studied compounds. The results of this study can aid designing of novel TACE inhibitors useful in inflammatory diseases.

Keywords: tumor necrosis factor-alpha, 3D-QSAR, partial least square, principle component analysis.
Title:
Inulinase production, engineering and immobilization: an overview

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Abstract:
Inulinases are β-D-Fructofuranosidase which cleaves inulin and oligofructose to yield fructose. They are widely used to produce high fructose syrup (HFS) from inulin (linear chain of β2→1-D-fructofuranose molecules terminated by a sucrose residue). High fructose syrup is used in the food and pharmaceutical industry since fructose is considered the sweetest of all natural carbohydrates. Aspergillus strains were examined for producing of the desired enzyme and A. awamori 16877 and A. niger 5012 were resulted with the higher yields. The enzyme from 16877 was purified from the culture medium of A. awamori by filtration, ammonium sulfate precipitation, dialysis and affinity chromatography on ConA-Sepharose 4B. The enzyme was purified 74.38 folds with a specific activity of 278.96 U/mg then characterized. The endoinulinase gene was amplified and the amplified fragment was cloned in E.coli DH5α using pET26 as expression vector and expressed in E. coli Bl21 (DE3). The structural, functional and storage thermostabilization through the semi-rational modification of surface-accessible lysine residues by pyridoxal-5'-phosphate (PLP) and ascorbate reduction has been also explored. The obtained thermostable endoinulinase was characterized by differential scanning calorimetry (DSC), circular dichroism (CD) analysis. The kinetics and thermodynamics of irreversible thermal denaturation at various temperatures (25-60°C) also were studied. Moreover, molecular dynamics simulations using the GROMACS program and molecular docking using the LIGPLOT program enabled us to simulate PLP-modified species of the enzyme to identify possible representative modification-originated intramolecular contacts (e.g., intramolecular interactions between covalently attached PLP-Lys381 with Arg526 and Ser376) to explain the improved structural functional and storage thermostabilization of the endoinulinase. Solvent polarity based immobilization of the Inulinases are also discussed.

Keywords: inulinase; Inulin; high fructose syrup; chemical modification; domain engineering
Title:
Apoptosome formation revealed by split luciferase complementation assay

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Abstract:
A novel method based on split luciferase complementarity assay is used to monitor apoptosome formation. Apoptosome formation is the main step in progress of intrinsic pathway of apoptosis triggered by release of cytochrome c from mitochondria followed by oligomerization of Apaf-1 monomers. Moreover, it has been suggested that release of cytochrome c from mitochondria and its binding to the WD-40 repeats of Apaf-1 causes conformational changes which initiate apoptosome formation. Although there is significant experimental support for apoptosome formation, but its detail structure within living cells are not clearly known. In order to direct confirmation of this model and also earlier detection of apoptosis, a novel method using a split luciferase complementation assay is designed based on oligomerization of N-luc-Apaf-1 and C-luc-Apaf-1 monomers. Our data here support a direct evidence for oligomerization of Apaf-1 molecules through their CARD domains and apoptosome formation following apoptosis induction at cellular level. Moreover, apoptosome formation occurs about 5 hours earlier than the appearance of significant caspase3/7 activity upon induction of apoptosis by doxorubicin. Time-response curve of split luciferase present a sigmoidal behavior which may indicate cooperativity in oligomerization of Apaf-1 upon binding of cytochrome c

Keywords: split luciferase complementary assay, apoptosome formation, binding
Title:
DNA Conformational Changes Induced by Natural Carotenoids and Monoterpene Aldehydes

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Abstract:
The effect of various carotenoids and monoterpene aldehydes, extracted and purified from saffron, on DNA structure is reported here. These components bind to the minor groove of high molecular weight DNA and at higher concentrations induce B-DNA to C-DNA transition. They also show different affinity for A.T or G.C. oligonucleotides; so that safranal induces double strand to triple strand (H-DNA) transition only on G.C. oligonucleotides which is in accordance with its comutagenic activity. In addition, the affinity of various carotenoids and monoterpene aldehydes for G-DNA or I-Motif, as two important DNA tetraplexes, are different. Furthermore, these natural ligands affect the structure of many proteins including histoen H1, albumin, hemoglobin, alfa-crystallin, catalase, collagen, etc. Therefore, both DNA-protein and protein-protein interactions are changed in the presence of the mentioned ligands. All the named activities together with their anti-oxidant, anti-inflammatory, anti-glycating, anti-aggregating and some other properties cause that they show many biological activities that induce apoptosis in the cancerous cells and protect the cells from apoptosis in the central nervous system. The molecular mechanisms involved in the mentioned changes are investigating by us in the cancerous cell lines; animal models of cancers, diabetes and chronic neuropathic pain; as well as in the test tube using various biochemical, biophysical and molecular biology techniques.

Keywords: Saffron, Carotenoides, Monoterpene Aldehydes, DNA Structure, Protein Conformational Change.
Title: Polyaniline Nanocomposites: From Chemical Sensors to Biochemical Sensors

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Abstract: A large number of chemical sensors have been developed using conducting polymers. They have attracted interest due to their combined properties of organic polymers and electronic properties of semiconductors. They offer great design flexibility, have high sensitivities, short response time and operate at room temperature. Among the conducting class of polymers, polyaniline (PANI) is one of the most attractive conducting polymer that has been extensively used as chemical sensor. It is unique due to its relatively facile synthesis, conductivity and environmental stability. However, poor processability of PANI and lack of selectivity has greatly restricted its use in commercial sensors. This limitation have been overcome by developing nanostructured PANI. In nanosize, they can be dispersed easily in a medium. Nanoscaled forms of PANI are better in performance because of their high surface area and faster response. Similarly, the problem of selectivity have been resolved by synthesizing hybrid nanomaterials with functional properties. New performance characteristics are achieved by combining properties from the inorganic and polymer components. Nanocomposites of PANI with metals, metal oxides, ceramics etc. are of current interest because of their multifunctionality, ease of processability, potential for large scale manufacturing, significantly lighter materials, higher selectivity and stability. In addition to this, owing to its biocompatibility and inherent electroactivity, PANI acts as a suitable matrix for immobilization of biomolecules. Nanocomposite biosensors prepared by using PANI as a support material exhibit fast response times and high storage and operational stability.

Keywords: None
Title: An improved hidden Markov model for 7TM protein topology prediction

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Abstract: Introduction: Knowledge about the topology of G protein-coupled receptors (GPCRs) can be very useful in predicting diverse range of properties about these proteins, such as function, three dimensional structure, and ligand binding site. Considering that only few GPCRs have known structures, large amount of computational efforts have been carried out to develop methods for predicting their topology. Materials and Methods: A novel method to predict the location and the length of transmembrane helices in GPCRs was proposed. This method consists of a “one by one” amino acid feature extraction window which makes it possible for the method to learn the amino acid distribution in helical segments of GPCR proteins. It is based on hidden Markov model (HMM) with a specific architecture that takes advantage of Viterbi decoding algorithm and the observed frequency values for adjusting the transition probabilities. Results: The prediction capability of the method was evaluated for per-protein, per-segment and per-residue accuracies on two datasets consisting of 649 (at least one GPCR from each family) and 2898 (all GPCRs) sequences extracted from UniProt database and compared with other commonly used methods. It was found that in all three assessments, the prediction accuracies for the new method on the larger dataset, i.e., 2898 GPCRs, were higher than that obtained by other methods. Conclusion: The results showed that our method was able to predict the topology of GPCR proteins without any sequence length limitation with the accuracies of 88.9% and 87.4% for the small (i.e., 649 GPCRs) and large (i.e., 2898 GPCRs) datasets, respectively.

Keywords: Hidden markov model, viterbi algorithm, G-Protein Coupled Receptors, Topoly prediction, TM helices.
Title:
Three Decades Differential Scanning Calorimetry Research on Proteins in IBB

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Abstract:
Biological activity of protein depends on their proper structure and stability. Study on protein stability has attracted many scientists. There are various methods to drive the thermodynamic parameters of proteins. Differential Scanning Calorimetry (DSC) is unique and powerful tool which directly measures the thermodynamic parameters of proteins. Ordinary, thermodynamic parameters are driven based on assumption that there is equilibrium between folded and unfolded state of protein. It is well known that this hypothesis is not valid for most of proteins and their unfolding is an irreversible process. From this point of view protein unfolding is kinetically controlled process. For irreversible proteins, DSC thermograms are obtained at different scanning rates and protein concentrations. Then curves are analyzed by fitting the data to theoretical equations for the dependence of the excess heat capacity on temperature. In our lecture will have an overview on three decades DSC research on different proteins in Biophysical Chemistry Lab (www.ibb.ut.ac.ir/bcl) at IBB.

Keywords: Biological activity, Differential Scanning Calorimetry, irreversible proteins
Title: A new Strategy based on pharmacophore-based virtual screening in adenosine deaminase inhibitors detection and in-vitro study

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Abstract: Background and the purpose of the study: Adenosine deaminase (ADA) inhibition not only may be applied for the treatment of ischemic injury, hypertension, lymphomas and leukaemia, but also they have been considered as anti-inflammatory drugs. On the other hand according to literatures, ADA inhibitors without a nucleoside framework would improve pharmacokinetics and emitted toxicity. Hence we have carried out a rational pharmacophore design for non-nucleoside inhibitors filtration. Methods: A merged pharmacophore model based on the most potent non-nucleoside inhibit or erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) and natural products were generated and applied for compounds filtration. The effects of filtered compounds based on pharmacophore and docking studies investigated on ADA by UV and fluorescence spectroscopy techniques. Results: Among 500 compounds three of them subjected for biological tests. filtered compounds were find efficiently inhibit ADA, and the most potent one shows an inhibition constant equal to 20 µM. Besides, fluorescence spectroscopy studies revealed that enzyme 3D structure bear further change in lower concentrations of that compound. Conclusion: 3 non-nucleoside inhibitors for ADA are presented. According to obtained results from UV and fluorescence spectroscopy, such interesting pharmacophore template with multiple approaches will help us to extract or design compound with desired properties.

Keywords: Adenosine deaminase, Pharmacophore Docking, Lead discovery, inhibitor
Title:
A Preliminary Investigation of the Jack-Bean Urease Inhibition by Randomly Selected Traditionally Used Herbal Medicine

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Abstract:
Background and the purpose of the study: Helicobacter pylori (H. pylori) infection leads to different clinical and pathological outcomes in humans, including chronic gastritis, peptic ulcer disease and gastric neoplasia and even gastric cancer and its eradication depends upon multi-drug therapy. The most effective therapy is still unknown and prompts people to make great efforts to find better and more modern natural or synthetic anti-H. pylori agents. Methods: In this report 21 randomly selected herbal methanolic extracts were evaluated for their effect on inhibition of Jack-bean urease using the indophenol method as described by Weatherburn. The inhibition potency was measured by UV spectroscopy technique at 630 nm which attributes to released ammonium. Results: Among these extracts, five showed potent inhibitory activities with IC50 ranges of 18-35 µg/mL. These plants are Matricaria disciforme (IC50=35 µg/mL), Nasturtium officinal (IC50=18 µg/mL), Punica granatum (IC50=30 µg/mL), Camelia sinensis (IC50=35 µg/mL), Citrus aurantifolia (IC50=28 µg/mL). Conclusion: Medicinal plants, traditional medicinal and other natural sources are still good source for lead discovery. The results of this study revealed that random screening of medicinal plants could lead to introducing new candidate for further studies which, in the end, can help and enhance human health.

Keywords: Herbal extract, Urease; Inhibitor, Indophenol method, Lead discovery
Title:
Coenzyme Q10 supplementation on metabolic status of type 2 diabetic patients

Authors:
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Abstract:
Introduction: Increased oxidative stress and impaired antioxidant defense contribute to pathogenesis and progression of type 2 diabetes. Consistent with this fact, it has been shown that diabetic patients have reduced coenzyme Q10 level. In this study we sought to compare the effect of coenzyme Q10 versus placebo on glycemic control and lipid profile in type 2 diabetic patients. Methods: In a randomized double-blind placebo-controlled trial, 64 type 2 diabetic patients were randomly assigned to receive either 200 mg Q10 or placebo daily for 12 weeks. Fasting blood samples were obtained and fasting plasma glucose (FPG), HbA1c, total cholesterol (TC), triglycerides (TG), LDL-C and HDL-C were measured. Results: In this study no significant differences considering age, body mass index (BMI), diabetes duration, FPG, HbA1c, TC, TG, LDL-C and HDL-C were shown between two groups. Serum HbA1C concentration decreased in the Q10 treated group (8±2.28 vs. 8.61±2.47%) with no significant effect in the placebo group. Following intervention no differences have been shown regarding FPG, TG and HDL-C in Q10 treated group. Furthermore, mean differences of TC and LDL-C level were statistically altered between two groups (P-value =0.027 and 0.039 respectively). Conclusion: In this study, Q10 treatment improved glycemic control, total and LDL cholesterol but these differences were associated with no favourable effects on TG and HDL-C.

Keywords: Glycemic control; Lipid profile; Oxidative stress; Q10; Type 2 diabetes mellitus
Title: Crude oil contaminated soil effects on catalase activity in Lentiles shoot

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Abstract: Catalase is an enzyme, which catalyzes the decomposition of hydrogen peroxide to water and oxygen. In stress conditions this enzyme is really helpful. Environmental pollutions such as crude oil contaminated soil pollution can put plants in stress condition. In this case Catalase is essential for plants to remove free radicals from the cells. Here we study the effect of crude oil on Catalase activity in lentile. Material and methods: Lentils were planted in crude oil contaminated soil and after 30 days they were cultivated. Plants shoots were cutted into small pieces and cells hemogenyzed with ultrasonic. After centrifugision, the upper solution were used as cellular soup. In this study kinetical factors, effect of pH and effect temperature on Catalase were measured and compared with control samples. Results: kinetical factors in control and test samples were different as activity in test shoot was 0.02 U/mg protein also Vmax in test shoot was 0.47 mM/min mg protein and in control shoot was 1.42 mM/min mg protein. Although the best temperature for both groups were 30 c and the best pH for both groups were pH=10 but curve pattern in two groups were different. Discussion: Presence of crude oil in soil as a factor of stress can effects on catalase activity in shoot and changes kinetical factors.

Keywords: Catalase, Lentil, Kinetical factors, pH, Temperature
Title:
Prevention of amyloid aggregation by a chemical analog of curcumin and its possible molecular mechanism inhibition

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Abstract:
Introduction: Protein aggregation is associated with a variety of pathological conditions, including Alzheimer’s and Parkinson diseases. The identification of aggregation inhibitors are fundamental in the quest to mitigate the pathological consequences. Recent studies have shown that Curcumin is capable to reduce amyloid plaques. Based on this bioactivity, we hypothesized that some curcumin analog also can inhibit amyloid aggregation. Several observations demonstrating that these reagents due to interfering in the formation amyloidal core via π-stacking interactions are capable to inhibiting amyloid fibril formation. In the present study, the inhibitory effect of 2,6-DI VANILLYLIDENE CYCLOHEXANONE on amyloid fibrillation of hen egg white lysozyme (HEWL) was reported. Methods: Acidic pH and high temperature were used to drive HEWL toward amyloid formation. Various techniques including ThT and Congo red assay and far-UV CD, were employed to characterize the HEWL fibrillation process. Results: To determine whether the compound has any affect on the HEWL amyloid formation, it was added to the incubation medium and results showed that it has a significant effect in decreasing ThT intensity and also AFM micrograph showed that the reagent significantly inhibit amyloid fibrillation. Intrinsic and ANS florescence experiment showed that it could not protect the HEWL native state from conformational changes, but was effective in diminishing HEWL amyloid fibril formation, delaying both the nucleation and elongation phases. Conclusions: The present investigation therefore demonstrated that the curcumin analog mentioned above with disruption of π-stacking forces between amino acids in core of amyloid is capable to inhibition of the process.

Keywords: Amyloid aggregation, π-stacking forces, curcumin analog
Title:
Kinetics effect of methocarbamol on yeast sucrase activity

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Abstract:
Methocarbamol (Guaiacol glyceryl ether carbamate) is a skeletal muscle relaxant and widely used to relief the pain in muscles. Many drugs may have an interaction with each other when used at the same time. Sucrase is a hydrolytic enzyme that breaks down sucrose to its monomers, glucose and fructose. Yeast sucrase is taken as drug by patients with the Congenital Sucrease-Isomaltase Deficiency (CSID). In this study the interaction between methocarbamol and yeast sucrase was investigated. Our results showed that methocarbamol could inhibit sucrase activity. Different concentrations of drug had no effect on Km of enzyme while the Vmax reduced by increasing drug concentration. Double reciprocal plot showed that the drug has inhibited enzyme by non-competitive pattern. Measurement of IC50 (13 mM) and Ki (12 mM) of the drug revealed that methocarbamol did not bind to enzyme with high affinity. Fluorescence measurement showed that the drug binds to free enzyme and enzyme-substrate complex that were accompanied by structural changes and hyperchromicity on the enzyme.

Keywords: Drug, enzyme, inhibition, methocarbamol, non-competitive, sucrase
Title: Study of kinetics effect of cimetidine on alkaline phosphatase of Pseudomonas aeruginosa

Authors: Mitra Asghari1, Majid Rajabian1, Dariush Minai-Tehrani2

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Abstract: Alkaline phosphatase (ALP) is a hydrolytic enzyme responsible for removal of phosphate from various substrates. In human, the activity of serum ALP increases in patient with liver malfunction. In bacteria, this enzyme does not well express in the phosphate rich culture medium, while it will be produced by the cell in poor phosphate medium. Pseudomonas aeruginosa is a Gram-negative bacterium that has shown to be resistance to many antibiotics. This resistance makes the bacterium very harmful in some diseases. Cimetidine is an antagonist of histamine H2-receptor that inhibits the production of gastric acid. Cimetidine is used for the treatment of gastrointestinal diseases. In this study the inhibitory effect of cimetidine on Pseudomonas ALP activity was investigated. The bacteria were cultured in minimal salt medium with 1% ethanol as carbon source. The cells were harvested and broken by ultrasonic equipment. The cell free extract was used for enzyme assay. Our results showed that cimetidine can inhibit ALP by non-competitive manner. In the presence of different concentrations of drug, the Km (0.23 mM) of enzyme did not change, while the Vmax reduced by increasing the drug concentration. The Ki of drug was estimated to be about 0.61 mM which determined that cimetidine binds to enzyme with low affinity. Fluorescence spectrometry has shown that the binding cimetidine to enzyme could induce red shift and hypochromicity in emission peak of enzyme. This shift and quenching suggested that the tryptophan residues in enzyme enter to more polar medium after binding of drug to ALP.

Keywords: Drug, enzyme, inhibition, non-competitive, Cimetidine, Pseudomonas aeruginosa
Title:
poly(caprolactone) nanofibers fabrication with different roughness to tissue engineering

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Abstract:
Developing scaffolds that mimic the architecture of tissue at the nanoscale is one of the major challenges in the field of tissue engineering. Extracellular matrix fibers (ECM) are an important component in tissue and organ development. Nanofiber polymers could play the same critical role in tissue regeneration Process. Nanofiber polymers which compose scaffolds were investigated for potential application of skin tissue engineering. The present study reports the fabrication of poly(caprolactone) (PCL) scaffolds with different surface roughness via electrospinning that characterized by scanning electron microscopy. Fibroblast cells behavior on scaffolds was studied by MTT assay and dapi. Herein, we hypothesized that using the PCL scaffolds with different surface roughness drives fibroblast cell into a morphology that induced cell behaviors modulation that yield maximum amount of attachment and proliferation. So based on the need, the proposed fiber skin substitutes can be successfully fabricated and optimized for skin fibroblast attachment and growth. Promising strategies are currently being investigated to allow for the fabrication of optimal polymer nanofiber tissue engineering scaffolds with the goal of treating damaged and degenerated tissues.

Keywords: Electrospining, PCL, Tissue engineering, Scaffold, Nanofiber
Title:
PLGA substrate nanostructured prepared through electrospinning and freeze drying: cell attachment study

Authors:
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Abstract:
The aim of Tissue engineering is to repair, replace, maintain or improve the function of damaged tissue or organ. It will be available the novel hopes for treating mankind. Electrospun nanofibers are able to provide a three-dimensional environment to mimic the natural extracellular matrix (ECM) therefore play an essential role in regenerative medicine and tissue engineering. In this study we demonstrated the importance of scaffold fabrication method and also the effect of nanotopography to support the growth of cells. Two scaffolds was perpetrated base on the biodegradable and biocompatible poly (Lactid-Co-Glicolid) acid (PLGA) polymer by electrospinning and freeze dryer techniques and investigated their effectiveness in providing support for cell behavior. The scaffold structures were compared by use of a SEM. fibroblast cell culture test was done to investigate cell behavior and followed by inverted microscopic images. To investigate the effect of scaffolds on cell proliferation, we was done cell attachment experiment, DAPI staining, MTT assay using of fibroblast cell culture and followed by inverted microscopic images. Our results showed that electrospinning PLGA scaffolds compared with freeze-drying PLGA scaffolds provide the suitable surface for cell function. It seems that is because of similarity to the features to the native ECM.

Keywords: Tissue engineering, Nanofiber, PLGA, Electrospinning, Freeze- drying.
Abstract:
Phytochelatins are small metal-binding peptides that synthesized by phytochelatin synthase (gamma-glutamyl cysteinyl transference). This peptides are finding in different organisms such as Plant, Algae, Yeast, Bacteria, cyanobacteria and etc. because of their significantly importance accumulation and detoxification of heavy metal in nature and environmental cleanup technology and also great potential in genetic engineering usage. In this study we have analyzed 31 phytochelatin synthase amino acid sequences with MEGA5 software with Calculation of bootstrapped consensus phylogeny tree based on multiple alignment sequences with 1000 replicates according to Neighbor-joining method and P-distance and sum branch of length(SBL), Molecular clock test and its tree topology, Best fit substitution model with some of statistical parameters like BIC (Bayesian information criterion), ALCc (Akaike Information Criterion, corrected), Maximum Likelihood value (lnL), an amino acid frequencies, Homogeneity of substitution pattern with calculation of Disparity index for per site, Monte Carlo test (500 replicates) for estimate the P-values. In conclusion Null hypothesis of equal evolutionary tree was rejected at 5% significant level (p<5.7067e-122) and WAG+G model has estimated as best fit substitution model.

Keywords: phytochelatin, phylogeny tree, molecular clock, BIC
Title: Three Dimension Structures Prediction of t-anethole Pathway Enzymes Using Homology Modeling

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Abstract: Anol synthase is multifunctional and NADPH dependent enzyme that can catalyze the conversion of coumaryl acetate into anol enzyme that transcript from AIS1 gene, also another enzyme in this pathway, t-anol/isoeugenol O-methyltransferase that catalyzed t-anethole synthesis reaction that transcript by AIMT1gene, t-anethole (1-methoxy-4-[(1E)-prop-1-en-1-yl]benzene), is one of The Phenylpropanoid Derivatives of secondary metabolism that find in Foeniculum vulgare (bitter fennel) and Pimpinella anisum. We have obtained amino acid sequences of isoeugenol O-methyltransferase and anol synthase from Uniprat database and we have used PYRE2 software for predicting Tertiary and secondary structure and disorder based on Homology modeling algorithm of this enzymes with detecting sequence homologues with PSI-BLAST and scanning constructing Hidden Markove Model (HMM) this sequence with homologues detecting and constructing 3D model for sequence of this protein Based on the alignments between the HMM of query sequences and the HMMs of known structure with accuracy Based on core of the protein within 2-4 Å RMSD (Root Mean Square Deviation) from the native structure, secondary structure predicted at α-helix, β-strand, coil and disorder levels with their SS confidence line that indicates the confidence in the prediction, and with Average 78-80% accuracy (i.e. 78-80% of the residues are predicted to be in their correct state) and also domain analysis is done. In conclusion 3D structure is predicted and displayed.

Keywords: HMM, PYRE2, RMSD
Title:
Computational Nano Study on Structure and Dynamics of Na+ and Ca2+ channels proteins

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Abstract:
The voltage gated sodium is a useful drug target and it is important physiologically. It was first discovered in 1980. Its structure similar to voltage gated potassium channels. Recently, the crystal structure for the voltage gated sodium channel was discovered and published. Voltage gated sodium channels are integral to the nervous system. They are crucial to the generation of nerve impulses. Its structure is similar to that of most other voltage gated ion channels: subunits arranged in such a way so that a central pore is formed. Ions travel through the pore on electrochemical gradients. We used the methods Molecular Dynamics, Langevin Dynamics, Monte Carlo, single point and Geometry optimization and The force fields are MM, AMBER, BIO and OPLS and different temperatures. By these methods were evaluated and significant results were obtained. That the energy obtained, the study examined and compared to simulation with empirical studies.

Keywords: voltage gated ion channels
Title: Artemisinin Drug Delivery Using Multiple Surfactants and Evaluation of Their Efficiencies in Different Cell Lines

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Abstract: The aim of this study was to investigate the niosomal artemisinin preparation, and its application on breast cancer cell lines. Artemisinin is a botanical anticancer drug which used in chemotherapy. Surfactants stabilize the emulsion by reduction of the surface tension and production of electrical and mechanical impediments. Non-ionic surfactants were used for production of niosomes. When compatible surfactants were mixed to each other results in stable emulsion. Niosomes are provided through reverse phase evaporation method with different proportions of span60/tween60/PEG600/H2O. Zeta sizer and TEM are used for measuring the average size and morphology of niosomes, respectively. In order to determine the entrapment efficiency, 1 mg of each formulation were centrifuged for 30 minutes at 4°C and at 50000 rpm. After that, optical density of the supernatant of each formulation were determined at 195 nm by means of spectrophotometer. Encapsulation efficiency was calculated by dividing of entrapped drug by total drug in one milligram of formulation multiply in 100. The cytotoxicity effect of different formulations was inspected by MTT assay on MCF-7, C6 and T47D cell lines. The results showed that encapsulation efficiency and cytotoxicity effects of multiple surfactants (stated as IC50) has better results than single surfactant in preparation of niosomal drugs.

Keywords: Artemisinin, Niososome, mixed surfactant MCF-7, C6, T47D, Cytotoxicity
Title:
The Possible Impact of Obesity on Androgen, Progesterone and Estrogen Receptors (ERα and ERβ) Gene Expression in Breast Cancer Patients

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Abstract:
Introduction: Obesity has been associated with increased mortality from hormone dependant cancers such as breast cancer which is the most prevalent cancer in women. The link between obesity and breast cancer can be attributed to excess estrogen produced through aromatization in adipose tissue. The role of steroid hormone receptors in breast cancer development is well studied but how obesity can affect the expression pattern of steroid hormones in patients with different grades of breast cancer was the aim of this study. Methods: In this case-control study, 70 women with breast cancer participated with different grades of obesity (36 none obese, BMI < 25 kg/m2 and 34 obese, BMI ≥ 25 kg/m2). The mean age of participants was 44.53 ± 1.79 yr (21–70 yr). The serum level of estrogen, progesterone and androgen determined by ELISA. Following quantitative expression of steroid hormone receptors mRNA in tumor tissues evaluated by Real-time PCR. Patients with previous history of radiotherapy or chemotherapy were excluded. SPSS 16 was used for data analysis and P < 0.05 considered statistically significant. Results: The difference in ERα, ERβ and PR mRNA level between normal and obese patients was significant (P <0.001). In addition, the expression of AR mRNA was found to be higher than other steroid receptors. There was no significant relation between ERβ gene expression in two groups (P = 0.68). We observed a significant relationship between ERα and AR mRNA with tumor stage and tumor grade, respectively (P = 0.023, P = 0.015). Conclusion: According to the obtained results, it is speculated that obesity could play a significant role in estrogen receptors gene expression and also could affect progression and proliferation of breast cancer cells.

Keywords: obesity, breast cancer, steroid receptors, steroid hormones
Title:
Structural and functional survey on interaction of human hemoglobin with n-alkyl sulfates homologues

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Abstract:
Introduction: The scientific study of protein surfactant interactions goes back more than a century. As properties of these interactions are so interesting to scientist, the research has been continued to date. The amphiphilicity of surfactants makes them a good model to investigate the interaction of hydrophobicity and hydrophilicity of them with different proteins. Now the question is how the relationship between the hydrophobicity in a homologue n-alkyl series and structural and functional changes of hemoglobin. Method: In this study spectroscopic techniques including UV-Vis, flourescence spectroscopy and oxygen affinity sensor were applied. Results: In this report, the interaction of hemoglobin with homologues of sodium n-alkyl sulfates with different chain length under physiological condition was assessed to find out the effect of change in hydrophobicity of ligand on the hemoglobin and found the changes in protein structure. Hemoglobin UV-Vis spectra show five peaks in different wavelength and these homologue n-alkyl sulfates can affect all of them. The flourescence spectrum showed third structural change according to alkyl chain length. Oxygen affinity changes upon interaction of these hemologues n-alkyl sulfates with hemoglobin. Conclusions: The increase of hydrophobicity in amphiphilic additives can affect structure and function of hemoglobin.

Keywords: n-Alkyl sulfates, Hemoglobin, Spectroscopy
Title:
Effect of Low-Level Laser on Erosive Oral Lichen Planus

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Iran. Tabriz, valiasr, homafar, Sadeghiyan Ave. No : 6

Abstract:
Introduction: Oral lichen planus (OLP) is an inflammatory disease that can be painful, mainly in the atrophic and erosive forms. Treatment of OLP remains a great challenge for clinicians. No therapy for OLP is completely curative; the goal of treatment for symptomatic patients is palliation. This study aimed to evaluate the use of low-level laser radiation for the treatment of symptomatic OLP. Method: To find out the indications and positive points of laser therapy in oral lichen planus, a search was performed in MEDLINE, PUBMED articles from 2007-2012. Results: The size of lesions, visual analogue score of pain, and stability of the obtained result in the follow-up period were evaluated in lichen planus patients. Also the effect of low intensity laser therapy was compared with topical corticosteroids. Conclusion: Studies detailed significant reduction in lesion size and pain. No reported complications or therapy side effects were observed in any of the patients treated.

Keywords: Key Words: Erosive OLP, Low-level laser radiation, Visual analogue score of pain.
Title:
siRNA transfection by Chitosan polysaccharide nanoparticle to inhibit influenza virus inhibition

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Abstract:
Anti-influenza chemical drugs affecting virus life cycle have always been applied for inhibition of the virus and consequently drug resistance is resulted. Interfering RNA has been studied recently to overcome this problem. RNAi are oligonucleotide sequences which are being applied for different objectives such as prevention of disease progression by inactivation of gene of interest e.g. cancer and viral genes. RNAi can specifically inhibit the function of inappropriate genes without any interfering in other genes. In this study, siRNA against enhanced green fluorescent protein (eGFP) and influenza virus nucleoprotein (NP) gene was used. Chitosan, a natural and non-toxic polysaccharide, was used as a vector to present siRNA effectively. Labeled siRNA was combined with chitosan nanoparticles and transfection of this compound into the cells was evaluated with immunofluorescent technique. In order to evaluate the inhibitory effect of this combination on protein expression, eGFP was transfected into cells. Chitosan loaded with anti eGFP – siRNA and siRNA against NP were transfected to the cells. The inhibitory effect of siRNA on eGFP expression was assessed using IF and flow cytometry. The viral replication inhibition was determined by hemagglutination assay. Our result revealed that transfection of siRNA in chitosan vector effectively inhibited the eGFP expression and the influenza virus replication.

Keywords: Chitosan, SiRNA, Influenza
Title: Analysis of Aldehydes in Cigarette Smoke and Injectable Formulations by Dispersive Liquid–Liquid Microextraction Combined with High Performance Liquid Chromatography

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Abstract: Introduction: The aldehydes are ubiquitous products produced from natural and industrial sources. These compounds exhibit potentially adverse health effects and are also recognized as biomarkers of cancer disease. Recently, a novel microextraction method named dispersive liquid–liquid microextraction (DLLME) has been developed, which is based on the use of an appropriate extraction and dispersive solvent. In this study, DLLME was combined with liquid chromatography (LC) to determine some aldehydes. Method: Firstly 2,4-dinitrophenylhydrazine and sulfuric acid were injected into the sample solution in the test tube. After heating at water bath, the volume made up to 10 mL. Then, 600 µL ethanol (as dispersive solvent) containing 50 µL CCl4 (as extraction solvent) was rapidly injected into the aqueous sample. Thus, the formed hydrazone was rapidly extracted into fine dispersed droplets. After centrifugation, the supernatant aqueous phase was readily decanted. Remained organic phase was diluted to 500 µL and a volume of 25 µL was injected into the LC system. Results: The effects of various experimental parameters on derivatization and extraction conditions were studied. Under the optimum conditions, calibration curves were linear in the range of 0.025 to 1.0 µg mL−1 with correlation coefficients of 0.9980 to 0.9996. Limit of detections were found to be 7.92–21.3 µg L−1. The relative standard deviations (RSDs) for inter– and intra–day assays were lower than 8.5%. Average recoveries for spiked samples were in the range of 86.0 to 109%. Conclusions: The proposed method gives a simple, relatively sensitive and low-cost procedure for the determination of aldehydes in cigarette smoke and injectable formulations. Sample preparation time as well as consumption of toxic organic solvents is avoided.

Keywords: Dispersive liquid–liquid microextraction, Aldehyde, Cigarette smoke, Injectable formulation, HPLC.
Title: Permeation of ions through ion channel

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Abstract: Introduction: Ion channels are pore-forming proteins that help the establishment and control the voltage gradient across the plasma membrane of cells by allowing the flow of ions down their electrochemical gradient. They are present in the membranes that surround all biological cells. They are responsible for processes such as the effluence of nerve impulses and muscle contraction. Due to the comparable size of CNTs and BNNTs to a biological ion channel, a CNT has been considered to have great potential for applications in biological nanosystems. The permeation of ions across membranes enclosing living cells is an essential procedure that controls the electrical properties of the cells, such as, the action potential generation in nerves and muscles. The cell membrane, however, is almost impassable to ions. Thus, an ion channel is needed to allow the transfer of ions in and out of the cell through the membranes. Method: We consider CNTs and BNNTs. The considered nanotubes were optimized in the B3LYP level of theory with the 6-31G** basis set implemented in Gaussian03. Molecular dynamics simulations were performed using NAMD. Results: In this work in order to obtain ionic current and ion-water RDF, the MD simulations have been performed. The results of MD simulations show that Mg2+ is permeated through (7, 7) nanotubes whiles Cl- is permeated through (8, 8) nanotubes. Conclusions: The selective ion permeation via carbon and boron nitride nanotubes has been investigated by MD simulations. It was shown that the ion permeation through considered nanotubes happens in the presence of electrical field and is also selective.

Keywords: Molecular Dynamics simulations, nanotube, preferential permeation.
Title:  
The Association of the Human Leukocyte Antigen (HLA) with the pathogenesis of Helicobacter pylori

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Abstract:  
Introduction: Urease as a main pathogenesis factor of H.pylori, attaches to HLA-II expressed on the surface of Gastric Epithelial Cells (GECs). There is a contribution of HLA-DQA1 gene to the host's response against H. pylori. It is suggested the HLA-DQA1 gene may contribute to the susceptibility or resistance to H. pylori infection. The current review aimed to evaluate the results obtained by different studies carried out so far on correlation of human HLA and the pathogenesis of the H. pylori in gastric epithelial cells. Methods: Related paper collected from valid data banks such as PUBMED and according to PRISMA diagram 2009, included and excluded papers selected. Review done based Matrix review system. Result: The frequency of HLA-DRB1*11,*14 and -DQB1*03 alleles were significantly lower in H. pylori–negative patients than in H. pylori–positive patients and HLA-DRB1*03 frequency significantly higher than in H. pylori–positive patients. Conclusion: This finding shown a relationship between the role of HLA in H.pylori infection therefore more studies are needed to clarify HLA-II polymorphism and its potential to treatment or prevention.

Keywords: H.pylori, HLA polymorphism, pathogenesis.
Title: The role of the dinuclear zinc-β-lactamase in catalyzing the β-lactams antibiotics

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Abstract: Introduction: β-lactam antibiotics, a major class of chemotherapeutic agents, used in the treatment of bacterial origin diseases. The most prevalent mechanism of bacterial resistance to β-lactam antibiotics is the production of β-lactamase, which inactivate these drugs by hydrolyzing the active β-lactam bond. Besides of many experimental and theoretical studies on the dinuclear zinc-β-lactamase enzyme have been reported, but the mechanisms of these enzymes are still not well understood. We focus on the CcrA enzyme, one of the four subclasses of metallo-β-lactamase (MβL), which contains two Zn2+ ions in their active sites. Method: Quantum mechanical calculations have been done with the Gaussian program series 2003 by using B3LYP method with four 3-21G, 6-31G, 6-31G* and 6-311G** basis sets. The solvent effects have been investigated with a PCM method for water solvent. Results: Calculations on the metallo-β-lactamase in complex with β-lactam antibiotics, shows that the catalytic activity occurs in two steps, nucleophilic attack of the bridging hydroxide ion on the substrate and protonation of the leaving amino group and also shows a significant role of the two zinc ions in catalysis. Conclusions: Zinc-β-lactamase enzyme plays vital biological roles in all organisms and are targets for a number of drugs and drug candidates and production of Zinc-β-lactamase most often renders bacteria resistant to almost all β-lactam drugs so far designed, in addition this enzyme can be used by the metals such as Co2+, Cd2+ instead of Zn2+. Due to limitations of computing systems, we have to use a model for dinuclear zinc-β-lactamase. Keywords: Dinuclear zinc-β-lactamase, Antibiotics, QM calculation.
Title:
Molecular mechanism of O-GlcNAcase using a fluorogenic GlcNAc substrate: A quantum mechanical approach

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Abstract:
Introduction: O-Glycoprotein 2-acetamino-2-deoxy-β-D-glucopyranosidase (O-GlcNAcase) is the enzyme responsible for the cleavage of the β-O-linked GlcNAc from the serine or threonine residues. O-GlcNAcase uses a "substrate participation", "anchimeric assistance" or "neighboring group participation" mechanism. Asp174 and Asp175 have been identified as the two key catalytic residues of human O-GlcNAcase. In the first step of the reaction, the cyclization step, Asp174 directs and polarizes the 2-acetamido group to act as a nucleophile and form the oxazoline intermediate. Asp175 meanwhile acts as a general acid, encouraging departure of the aglycone leaving group. To address the role of the 2-acetamido group of the substrate we used of 4-methyleumbelliferyl 2-N-acetyle-2-deoxy-β-D-glucopyranosidase (MUGlcNAc) as fluorogenic substrates model for O-GlcNAcase bearing differing levels of fluorine substitution on the N-acetyl group. Method: Ab initio calculations were carried out with the Gaussian program series 2003. All geometries were fully optimized employing B3LYP/6-31G* and B3LYP/6-311G** methods. QST2 method was used to search for transition state. The solvent effect on the conformational equilibrium has been investigated with a PCM method for water solvent. Results: We have applied QM calculations to study fluorine substituents effects on the N-acetyl group on an enzymatic reaction using anchimeric assistance. The energy profile indicates an exothermic chemical reaction. Conclusions: This report generates new theoretical treatments in drugs with sugar part which makes the interpretation of saccharide conformational analysis more feasible. We hope the results of this study propose new compounds as potent inhibitors to therapeutic diabetes and neurodegenerative.

Keywords: O-GlcNAcase, MUGlcNAc, Substrate participation, QM calculations.
Title:
Study of the Application of a Bacteriorhodopsin Monolayer on the Surface of Photocells

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Abstract:
Abstract: The goal of this study was to stabilize a monolayer of bacteriorhodopsin on titanium nanoparticle dioxide (TiO2) substrate cover of a photocell. A photocell can convert the sun’s radiation into electrical energy; pollution-free and with minimal cost. One of the best proteins for such an energy conversion system is bacteriorhodopsin with its proton production mechanisms. The TiO2 paste was synthesized on FTO glass surfaces, following Dr. Blading’s method. Next, the TiO2 substrate surface was modified by epoxy saline and glutaraldehyde to promote covalent interaction with bacteriorhodopsin. Molecular adhesion and aggregation of bacteriorhodopsin molecules were prevented in the lab, using SDS and Triton-X100 surfactants. Finally, the solution containing bacteriorhodopsin and surfactants were transferred over the TiO2 substrate. After 24 hours of incubation, the time required for stabilization of bacteriorhodopsin, the substrate surface was rinsed with the phosphate buffer. In conclusion, a monolayer of bacteriorhodopsin was covalently stabilized on the TiO2 substrate surface using a Self-Assembly Monolayer method. Our results indicate that the use of a bacteriorhodopsin monolayer, as compared to the use of the aggregated molecules, increased the electrical capacity of photocells 2.89 times.

Keywords: Monolayers bacteriorhodopsin, titanium dioxide (TiO2), glutaraldehyde, epoxy saline, SDS, Triton-X100 and Self-Assembled Monolayers.
Title:
Cytotoxic and apoptotic effects of Persian herbal plants against the fibrosarcoma (WEHI-164) cell line: in vitro analysis

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Abstract:
Abstract Background: Recently, Plant-derived extracts, as a Plant-derived chemotherapeutic anti-tumor drug introduce a new alternative source of effective cytotoxic anti-cancer agents against malignant tissues and cells due to their potential pharmacological activities. Ferulago Angulata DC (FA), Echinophora platyloba DC (EP), Salvia officinalis L (SO) and Chelidonium majus L (CM) plants from Persia were evaluated for their cytotoxicity and apoptotic effects on mouse fibrosarcoma cell line (WEHI-164) in comparison to mouse non-malignant cell line (L929).

Methods: Cytotoxic activity and cell viability of methanolic extracts were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and trypan-blue assay on WEHI-164 and L929. Cell death ELISA was employed to quantify the nucleosome production result from nuclear DNA fragmentation during apoptosis and determined whether the mechanism involves induction of apoptosis or necrosis. The cell death was identified as apoptosis using terminal deoxy-nucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay.

Results: The highest cytotoxic activity of extracts of Chelidonium majus L > Ferulago Angulata DC > Echinophora platyloba DC > Salvia officinalis L, respectively. Results were shown that these extracts decreased cell viability, inhibited cell proliferation, and induced cell death in a dose and time dependent manner, however did not exert any significant cytotoxic effect on mouse non-malignant cell line L929.

Conclusion: So the extracts C. majus, F. Angulata, E. platyloba, S. officinalis were found to selectively and dose-dependently inhibit the proliferation of fibrosarcoma cell possibly via an apoptosis-dependent pathway.

Keywords: crude extracts; cytotoxic activity; apoptosis; cancer; WEHI-164; herbal plants
Title:
Interaction of Gold Drugs with Proteins

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Abstract:
Gold compounds have been in use for treatment of rheumatoid arthritis for about 70 years but several questions relating to efficacy and toxicity still remain unanswered. The gold drugs include several injectable and the only oral compound auranofin [5,(1-thio-ß-D-glucopyranose-2,3,4,6-tetraacetato-S)-(triethylphosphine) gold(I)]. These are different chemical entities shown to have different in vivo chemistry and pharmacokinetics. Drug–protein interactions provide highly useful information for interpretation of pharmacokinetic parameters. Reactions of metal-based drugs with proteins are even more important with reference to their mode of action and toxicity. Interaction of aurothiomalate (an injectable drug) in vivo with plasma/serum proteins suggests that most of the gold is bound to albumin and a small fraction to immunoglobulins and low-molecular-weight substances. In humans the distribution after administration of auranofin was: 81.8% to albumin, 4.8% to α1-globulin, 6.9% to α2-globulin, and 6.5% to β and γ globulins at blood gold level of 1.5 µg mL⁻¹. The gold in erythrocyte membrane was initially very high and decreased rapidly afterwards with aurothiomalate, and auranofin produced constant high levels up to 36 weeks. A study with sodium aurothiomalate, gold keratinate, and triethylphosphine gold showed different distribution patterns. The aurothiomalate gold was 94% bound to albumin and only 6% to globulin, whereas in case of phosphine gold the corresponding figures were 70% and 30%, respectively. The affinity of keratinate gold to globulin was found to be high (20%). It appears that the affinity of gold to globulins changes with the nature of the drugs. These results provide understanding about different behaviour of sodium aurothiomalate and auranofin. These results can be used to design more effective and less toxic gold drugs.

Keywords: Chrysotherapy, Sodium aurothiomalate, Auranofin, Gold drugs, Drug-protein interaction
**Title:**
The Inhibitory effect of a new synthesized ligand on mushroom tyrosinase

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**Abstract:**
Introduction: Tyrosinase (EC 1.14.18.1) is a copper containing enzyme that is widely distributed in microorganisms, animals, and plants. Finding new tyrosinase inhibitors with low Ki values is very important because tyrosinase has a major role in both mammalian melanogenesis and enzymatic browning of fruit or fungi. Method: The inhibitory effect of (NZ)-N-[(4-methylphenyl)methylidene]-2-({2-[(Z)-[(4-methylphenyl)methylidene]amino]phenyl}disulfanyl)aniline on catecholase activity of both forms of mushroom tyrosinase (oxy form and met form) in a 10 mM phosphate buffer solution (pH 6.8), at 20°C with uv spectrophotometer was studied. L-Dopa was used as substrate of catecholase activity. Results: The results show that ligand competitively inhibits both forms of the enzyme with inhibition constants (Ki) of 1.4 and 0.8 µM for oxy and met forms respectively. For further insights the docking study between tyrosinase and ligand was done. The docking simulation showed that ligand binds in the active site of the enzyme near the Cu atoms and makes 2 hydrogen bonds with histidine and proline residues of active site. Conclusions: The Ki values for ligand for two different forms show that ligand has more tendency to bind met-form and also the Ki values show that it is a very potent.

**Keywords:** Mushroom Tyrosinase, Inhibition, catecholase, oxy form, met form
Title:
Novel biodegradable heparin-based nanocomposite system for targeted drug delivery against human ovarian cancer

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Abstract:
Introduction: Many heparin (HP)-drug conjugates have been developed for cancer chemotherapy as macromolecular prodrugs. Taking advantage of excellent properties of HP and polymer-drug conjugates, we have successfully synthesized a HP based SPIO NP drug conjugate, carrying two different anticancer drugs, DOX and PTX, for intratumoral drug delivery. The cytotoxicity response of the drug loaded HP-SPIO NPs was determined on the biochemical parameters and survival of human ovarian cancer cell lines of OVCAR-3 and A2780. Method: The bare (~10 nm) and heparin (HP)-coated superparamagnetic iron oxide nanoparticles (SPIO NPs; 42 nm) were formulated by co-precipitation technique. Results: The as-prepared HP-SPIO NPs had the saturation magnetization of 50-55 emu/g at 300 K. The anticancer drugs, doxorubicin and paclitaxel, were successfully partitioned in the SPIO core. Incubation with A2780 and OVCAR-3 human ovarian cancer cells revealed that the DOX-HP-SPIO NPs (85 nm) and PTX-HP-SPIO NPs (71 nm) showed sustained and pH-sensitive release of DOX (87%) and PTX (75%) at pH 6.0, even for up to 15 days. While, 5 µg/ml DOX-HP-SPIO NPs and PTX-HP-SPIO NP caused 93 and 87.1% apoptosis in A2780 and OVCAR-3 cells, respectively, with a sharp decrease in the level of bcl-2 and survivin proteins and increased expression of proapoptotic proteins, like bax and NF-κB. Conclusions: The presently formed nanocomposite-based drug delivery system was readily internalized into tumor cells and induced a higher apoptosis rate.

Keywords: Human ovarian cancer, Iron oxide, Heparin, Drug release, Loading efficiency, Apoptosis.
Title:
Docking study of DATAs derivatives as potent HIV-1 reverse transcriptase inhibitors

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Abstract:
Introduction: Diaryltriazine analogues (DATAs) are a class of interesting Non-nucleoside reverse transcriptase inhibitors which are highly effective against wild-type of HIV-1. Method: The experimental data comprising twenty five (IC50) values for wild type HIV-1, are collected from the literature. The molecular structures of the ligands are drawn using HyperChem software. Semi-empirical gas phase energy minimization is performed by the software. The PDB file of crystal structure of HIV-1 RT downloaded from PDB bank server (PDB code: 1JLQ) that is used in the docking simulations. Automated docking of the ligands is performed by the Autodock program. Results: We performed a test to ensure the validity of docking calculations. After validation, the docking was extended to the ligands. Docking studies on the DATAs derivatives showed that the binding pocket comprises of Pro95, Leu100, Lys101, Lys102, Lys103, Val106, ARG172, Lys173, Pro176, Asp177, ILE178, Val179, ILE180, Tyr181, Tyr188, Val189, Gly190, SER191, Phe227, Leu234, His235, Pro236, and Tyr318 of the p66 subunit and Glu138, THR139, Pro140 of the p51 subunit, which are mainly hydrophobic and aromatic residues. This program is also employed to determine the binding affinity of the DATAs derivatives toward the HIV1 RT. Conclusions: Using docking study, we have shown that all the studied DATAs derivatives dock into the HIV-1 RT and that have a common binding mode. The docking energies showed that averaging of the calculated docking energy and experimental log(IC50) in four groups of ligands result a good correlation which is representing that more potent ligands produce more negative binding free energy. The results from this study are now being used for the design of newer compounds with better anti HIV activity.

Keywords: reverse transcriptase, Diaryltriazine analogues, Molecular Docking
Title: Isolation and characterization of a new halo-thermo tolerant Bacillus from saline Lake of Iran in order to biotechnological applications

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Abstract: Introduction: Microbiological researches have revealed the presence of moderately to extremely halophilic microorganisms in saline environments. The aspects that attracted the attention of researchers were mainly those related to their physiological adaptation to highly saline concentrations. In this study, we report a new halo-thermotolerant Bacillus from a saline Lake of Iran with a practical perspective on biotechnology. Method: This Descriptive study was performed on water samples of Aran-Bidgol saline Lake that were collected in June 2011. Media for isolation of the bacteria were supplemented with 20% sea salts, yeast extract and tryptone. The isolate was identified by 16SrDNA analysis and biochemical characteristics. Also, biofilm formation, biosurfactant production and hydrolytic activates of isolate was evaluated. Results: Screening bacteria led to the identification of a new halo-thermotolerant Bacillus. On the basis of genetic and phenotypic data, this isolate was closely related to Bacillus licheniformis. But isolated Bacillus can be distinguished from B. licheniformis by salt tolerance, 16SrDNA sequence and some different physico-chemical properties. Optical density of eluted stain in 590 nm indicated strong biofilm formation (OD5901.25±0.31) for this Bacillus. Moreover, this microorganism exhibited highest activity for semi-quantities oil displacement test and evaluated positive for biosurfactant production. Amylase, protease and DNAase enzymes produced in present of 10-20% salt of medium. Conclusions: The present study could represent a new potential microbial source for remarkable bioenzymes, biosurfactant and exopolysaccharides in order to usage in bioremediation, medical and pharmaceutical industrial. Thus, the further studies will be necessary to determine the biotechnological applications of this organism.

Keywords: halo-thermotolerant Bacillus, phylogenetic analysis, biofilm formation, saline Lake
Title: Identification of miRNAs and their potential targets in halophyte plant Thellungiella halophila

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Abstract: microRNAs are a class of non-coding RNAs with important gene regulation roles in various organisms. to date, several plant miRNA have been deposited in the miRNA miRBase database (release 10.1). many of them are conserved during the evolution of land plants suggesting that the well-conserved miRNAs may also retain homologous target interactions. little is known about the experimental or computational identification of conserved miRNAs and their target genes in thellungiella halophila. here, Using a computational homology search approach and according to a series of filtering criteria, a total of 8 miRNAs belonging to 4 miRNA families were detected in the Expressed Sequence Tags (EST) databases. Then, potential target genes of predicted miRNA were subsequently predicted. our findings showed that the most of targeted gene were belonged to encode transcription factors and enzymes participating in regulation of development, growth and other physiological processes. this is the first in silico study of halophytes miRNAs which may help to understand the miRNA mediated regulation in high salinity conditions

Keywords: Thellungiella halophila, Expressed Sequence Tags, microRNA
Title:
Plant virus vectors as recombinant pharmaceutical expression systems

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Abstract:
Different expression systems for recombinant proteins production are known that including bacterial, yeast, transgenic plants, transgenic animals and mammalian cells. Among these systems, several vectors based on plant viruses were designed due to the development of new technologies. These expression systems employed to expression pharmaceutical proteins in non-transgenic plants. Advantages of these systems are potential for designing of different vector by modification of genes related to coated proteins and alternation of them with new genes, expression of diverse proteins at large -scale, safety and free of toxic and infectious agents for human, correct folding pattern and glycosylation and functionalized proteins. Novel strategies, magnification and agroinfection, have been utilized in plant hosts to express recombinant pharmaceutical proteins. Some of plant viruses that genetically manipulated for targeted proteins are BMV, TMV, PVX, PMMV, and OBDV. Several proteins were produced using these systems and introduced into clinical trial (phase I and II ), which involved H5N1 influenza antigen, LcrV (Yersinia pestis), human papillomavirus E7, Granulocyte-macrophage colony-stimulating factor (GM-CSF), Aprotinin, alpha galactosidase, monoclonal antibodies for non-Hodgkin’s lymphoma, somatotropin, single chain antibodies. To date, no reports are about adverse effects of clinical treatment of these products. This abstract summarized advantage of expression systems based on plant virus vectors to improvement it in our research laboratory.

Keywords: plant viruses, vectors, recombinant pharmaceutical proteins
Title:
Theoretical Study on the Microsolvation of Alanine at the X3LYP Level

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Abstract:
Introduction: Hydrogen bonding is widely studied in an array of chemical systems such as amino acids because they comprise a fundamental building block for biologically important molecules. This is due to the neutral/zwitterionic conformer possibilities that the amino and carboxylic acid functional groups provide. In this research we focused on the stability comparison between the neutral and zwitterionic forms of alanine. Theoretical model: In these studies which have been reported for alanine, the X3LYP functional resulted in hydration energy discrepancies of less than 3% when compared to CCSD(T) calculations using the same 6-311++G(d,p) basis set for the first and 6-311+G(2dp,p) for the second methods. Based on these reports, the geometry optimizations were carried out using the X3LYP hybrid functional with the 6-31G(d) and 6-31++G(d,p) basis sets in Gaussian 09 package. Results and discussion: All the structures of the neutral Ala, Ala–(H2O)n=1–5 and ZAla–(H2O)n=2–5 complexes have been optimized at X3LYP/6-311++G(d,p) level of theory. The binding energy of Ala–(H2O)n=1–5 complexes is calculated using the relationship: BE = Ecomplex _ (EAla + EH2O) and the values are calculated. The values of Gibbs energy and enthalpy of ZAla–(H2O)n=2–5 complexes are presented relative to the corresponding Ala–(H2O)n=2–5 complexes. The frequencies of hydrogen bond bridging modes, O--H and N--H have also been calculated. During the stepwise addition of water molecules it has been observed that the stable structure of ZAla–(H2O) complex does not exist in gas phase. We found that at least two water molecules are needed to stabilize the structure of ZAla in gas phase. It is also observed that the hydration process enhances the values of valence angle due to the redistribution and localization of charges among the water molecules. The Gibbs energies, interaction enthalpies and entropies of the complexes were also determined. The computed values of Gibbs energy and enthalpy are found to minimum for the ZAla–(H2O)5 complex which is contrast to similar reports. Atom in molecule (AIM) and natural bond orbital (NBO) methods have been used for hydrogen bond strength comparison and electrostatic charge changes during the hydrogen bond evolution.

Keywords: Alanine; DFT; Microsolvation; Hydrogen bonding
Title:
Investigation of pH effect on α1-antitrypsin structure via molecular dynamics simulation

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Abstract:
Introduction: Human α1-antitrypsin (AAT) is main member of serin protease inhibitors (serpin) super family which contains three β-sheet and nine α-helix and a reactive center loop. The important function of this inhibitor is inhibition of proteases. Structure information of this protein is important because it make us able to understand cause of malfunction of this protein and other serpins. Genetic and environmental factors such as temperature and pH can affect on the protein structure.

Method: In this study, the effect of pH on protein structure was investigated via molecular dynamics simulation. Simulation was performed using GROMACS 4.5.3 package. Molecular dynamics simulation was performed at pH 2.5 and 7 and 300 k for 20 ns in each pH.

Results: When the pH decrease from 7 to 2.5, the radius of gyration (Rg) of protein increase from 21.25±0.06 to 22.39±0.09 (Å), root mean square deviation (RMSD) increase from 1.26±0.09 to 4.72±0.83 (Å) and hydrophobic accessible surface area and total accessible surface area increase from 962±13 to 1058±16 and 1774±18 to 1916±23 (Å²) respectively. Also potential energy of protein increases from -1297519 to -1293969 (kj/mol) and the number of hydrogen bond in protein decrease from 300±9 to 262±7. In pH 2.5 surface accessible area of tryptophan 194 is almost constant and surface accessible of tryptophan 238 increased from 37.13±0.96 to 42.22±1.52 (Å²). The distance between carboxyl oxygen of glutamate 342 and amine nitrogen of lysine 290 was increased from 4.27±1.0 to 6.93±0.13 (Å).

Conclusions: Increase of Rg, RMSD, hydrophobic accessible surface area and total accessible surface area indicate unfolding of protein structure. Also increase of potential energy and decrease of number of hydrogen bond indicate instability of protein structure.

Keywords: Molecular dynamics simulation, α1-antitrypsin, pH
Title:
PTPN22 gene polymorphism C1858T is not associated with leprosy in Azerbaijan, northwest Iran

Authors:

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Abstract:
Introduction: Leprosy is a human chronic granulomatous infectious disease caused by Mycobacterium leprae. Several types of study support a role for host genetics in susceptibility to leprosy. The PTPN22 gene encodes an intracellular lymphoid-specific phosphatase (Lyp) that has been shown to play a negative regulatory role in T-cell activation. In the present study, for the first time in the world, we examined polymorphism in the PTPN22 C1858T (R620W) gene with respect to leprosy in a case-control study in the Azeri population of Northwest Iran. Method: One hundred and fifty-three treated leprosy patients and 197 healthy and ethnic matched controls were included in this study. Restricted fragment length polymorphism (RFLP) method was used to type PTPN22 C1858T polymorphism. Results: There was no significant difference in the distribution of the genotypes and allele frequencies of PTPN22 C1858T polymorphism between Leprosy patients and controls (P=641, and 0.645; respectively). Conclusions: In summary, the PTPN22 C1858T (R620W) is not relevant in susceptibility to leprosy in the Azeri population of Northwest Iran.

Keywords: leprosy, PTPN22, gene polymorphism.
Title:
PTPN22 gene polymorphism C1858T is not associated with type 1 diabetes in Azerbaijan, northwest Iran

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Abstract:
Introduction: Type 1 Diabetes (T1D) is a complex trait caused by T-cell mediated autoimmune destruction of islet beta cells in the pancreas, resulting from the interaction between genetic and environmental factors. The PTPN22 gene encodes an intracellular lymphoid-specific phosphatase (Lyp) that has been shown to play a negative regulatory role in T-cell activation. Several studies have shown that a functional mission PTPN22 C1858T (R620W) polymorphism confer susceptibility to several autoimmune diseases including T1D. In the present study, for the first time in Iran, we explored whether the PTPN22 C1858T (R620W) gene polymorphism confer susceptibility to T1D in the Azeri population from the Northwest region. Method: One hundred and fifty-six T1D patients and 197 healthy and ethnic matched controls were included in this study. Restricted fragment length polymorphism (RFLP) method was used to type PTPN22 C1858T polymorphism. Results: There was no significant difference in the distribution of the genotypes and allele frequencies of PTPN22 C1858T polymorphism between T1D patients and controls (P=0.840, and 0.842; respectively). Conclusions: In summary, the PTPN22 C1858T (R620W) is not relevant in susceptibility to T1D in the Azeri population of Northwest Iran.

Keywords: type 1 diabetes, PTPN22, gene polymorphism.
Title:
Quorum Sensing Inhibition of Berberis vulgaris L. var. Asperma on Streptococcus mutans: In Vitro Anti-Biofilm Activity and Molecular Docking Studies

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Abstract:
Introduction: Quorum sensing (QS) allows bacterial synchronization and downstream expression of associated virulence genes leading to efficient and effective colonization and survival within the host. The QS inhibition (QSI) capability of Berberis vulgaris L. var. Asperma berry (zereshk) extracts on Streptococcus mutans, an etiologic agent of dental caries and periodontitis, was investigated. Method: Biofilm inhibition at phenotypic and genotypic levels, liquid chromatography and mass spectrometry (LC-MS) analyses for phytochemical compounds including their putative roles were explored using AutoDock. Phenotypic biofilm inhibition was assayed by modified SYPRO Ruby fluorescence protocol while the genotypic assay was performed by quantification of expressed glucosyltransferase (gtf) transcripts by real-time quantitative PCR. Results: Both 20% and 50% ethanol extracts showed significant inhibition of biofilm formation at 99.53% and 99.55%, respectively (p <0.05), while significant down-regulation of gtf expression (p <0.05) was obtained at 99.98% for both. LC-MS analysis primarily identified chlorogenate, columbamine, rutin, isocorydine, rutin and trace levels of berberine, majority of which interacted efficiently with the bacterial communication QS regulator OmpP in S. mutans. Conclusions: These results indicate that bioactive compounds from B. vulgaris L. var. Asperma fruit has QSI activity against S. mutans biofilm formation and can potentially be used as an alternative or in combination with oral prophylaxis agents against dental caries and periodontitis.

Keywords: Berberis vulgaris, Streptococcus mutans, quorum sensing, anti-biofilm, glucosyl transferase
Title:
Anti-Inflammatory Compounds from Iranian Rhus coriaria L. (Somagh) Against Cytokines Expressed by Lipopolysaccharide-Induced Macrophage: Molecular Interactions with NF-kB

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Abstract:
Introduction: Anti-inflammatory agents allow proper control and regulation of the process of inflammation leading to less tissue damage evidenced by minimal clinical symptoms. The powdered dried fruits of Rhus coriaria L. (somagh) have long been used in Iran as an effective topical medicine to relieve painful gums and also reported to be a powerful antimicrobial, antifungal and anti-bleeding agent. The anti-inflammatory capability of somagh against expression of cytokines, IL-1β and TNF-α, was investigated.

Method: Aqueous and ethanolic extracts of somagh were tested for potential down-regulation of IL-1β and TNF-α transcripts in lipopolysaccharide-induced macrophage cultures in vitro. Levels of expressed cytokine transcripts were determined by real-time quantitative PCR. Phytochemical analysis of the extracts was examined by liquid chromatography and mass spectrometry (LC-MS). The putative roles of the major organic molecules identified where investigated by the molecular suite AutoDock. Biocompatibility of the extracts was also ascertained by PrestoBlue assay on normal human dermal fibroblasts (HDFn).

Results: Significantly down-regulated levels of both IL-1β and TNF-α were observed (p<0.05) using both aqueous and ethanol extracts starting from the lowest 10% concentration compared to controls. LC-MS chromatogram revealed major alkaloid phytochemicals majority of which significantly bind to the DNA binding site of NF-kB transcription factor. Both aqueous and ethanol extracts were also non-cytotoxic to HDFn.

Conclusions: These findings suggest the anti-inflammatory property of somagh as confirmed by down-regulation of cytokines in vitro and in silico binding and inhibition of NF-kB. The bioactive phytochemicals therein can be promising novel immunomodulatory drugs for dental and medical applications.

Keywords: Rhus coriaria, anti-inflammatory, cytokines, NF-kB
Title:
Effects of solution condition such as pH, Temperature and Salt concentration on Hen Egg White Lysozyme fibrillar aggregation

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Abstract:
Introduction: Protein aggregation is an important target in fibrillation mechanism and amyloid aggregation in brain and systemic diseases and is of prime importance. Human lysozyme (Hul) is an amyloidogenic protein that its mutant variants cause hereditary systemic amyloidosis. Since hen egg white lysozyme (HEWL) and Hul have a high degree of structural and sequential homology, it has been used as a model in studying Hul amyloidosis. Previous studies have shown that HEWL would form fibrils under high temperature and low pH. We applied different solution condition to follow HEWL aggregation behavior which comprise insights to fibrillation mechanism. Method: HEWL fibrillation was examined at different temperatures (37, 45, 54 and 70 °C), pH's (2, 4, 7, 8), and salt concentrations (0.1, 0.3 and 0.5 mM sodium chloride) by fluorescence and circular dichroism spectroscopies, and the images of aggregates (fibrillar and non-fibrillar) were obtained by atomic force microscopy (AFM) and transmission electron microscopy (TEM). Three dimensional structure of native HEWL was also reconstituted by PyMol 0.97 program. Results: HEWL fibril formation occurred by exerting the following conditions: temperatures close to or higher than protein unfolding temperature, i.e, 54 and 70 °C respectively, at pH 2, and in mixtures containing 0.3 mM sodium chloride. Conclusions: A general model was proposed for protein aggregation which ends up as amyloid fibrils, depending on environmental conditions and peculiarities of amino acid sequences. Our results confirmed that lysozyme can form fibrillar aggregates under harsh solution conditions and salt ions can promote amyloid fibril formation of HEWL. Since formation of partially unfolded conformations represents an important prerequisite for protein fibrillation, to propose a mechanism it is of great importance to pursue detailed fibrillation steps.

Keywords: Amyloid fibrils, Hen egg white lysozyme, Solution conditions
Title:
Thermodynamic study of complex between carbonic anhydrase enzyme and histamine: a quantum mechanical approach

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Abstract:
Introduction: Carbonic anhydrase (CA) is a zinc-enzyme widely spread in the animal, bacterial, vegetal and human which catalyzes one of the simplest physiological reactions: the reversible inter conversion between CO2 and HCO3¯ by a proton transfer (PT). Proton transport is an important event in many biological processes that influenced by environmental electrostatic.
Method: All calculations were performed using the Gaussian software. The geometries of carbonic anhydrase enzyme active site (CA), activator and its protonated form. The complex between activators and CA were fully optimized using DFT method with B3LYP functional. The calculations were performed with the standard 6-31G* basis set. The harmonic vibrational frequencies were computed to confirm that an optimized geometry correctly corresponds to a local minimum that has only real frequencies. Also the thermodynamic properties of all compounds were obtaine from frequency calculations at 298.15 K and 1.0 atmosphere pressure. The solvent effects on the conformational equilibrium and contribution to the total enthalpies were investigated with the PCM method at the B3LYP/6-31G* level. Results: The results of our calculation indicate that the histamine is a potent activator for CA enzyme. Histamine binds within the CA active site without interacting with the metal center directly.
Conclusion: This research can help for design Alzheimer’s drugs and other conditions in need of achieving spatial learning and memory therapy. Also to design the new potent activators.

Keywords: Carbonic anhydrase, Activator, Histamine, QM calculation.
Title: A simple procedure for estimation of the stoichiometry of surfactant binding to protein

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Abstract: Introduction: The most convenient method for the evaluation of the association of surfactants to proteins is equilibrium dialysis which is time consuming, and very expensive. Here we used the fluorescence spectroscopy as an alternative approach which is more sensitive, more convenient and needs lower protein contents. Method: Interaction of bovine serum albumin (BSA) with sodium dodecyl sulphate (SDS) was studied with fluorescence spectroscopy. The stoichiometry of binding was estimated from the following procedure: 1) a series of titrations were performed in presence of different protein concentrations, 2) the area below the fluorescence curves were plotted versus [SDS] which showed some transition points, 3) the total surfactant concentration at every transition point were plotted versus the total protein concentration. The number of bound surfactants per mole of protein(v) and [SDS]free simply were obtained from the slope and the intercept of the obtained trend line respectively applying the below equation: [SDS] total= [SDS] free + v [Protein] Results: The obtained binding isotherm of SDS to BSA had a good correlation with previous studies and showed the typical four characteristic regions of SDS binding to albumin. Conclusions: Though this method is not as straight forward as equilibrium dialysis and may miss to track some rare interactions that don’t alter fluorescence intensity, being simple, sensitive and cheap, this method has a good potential to be used broadly to study the surfactant protein interactions.

Keywords: sodium dodecyl sulphate, stoichiometry of binding, bovine serum albumin, fluorescence spectroscopy
Title: The role of Cu-Zn SOD in the protection of chs mutants of Arabidopsis plants against chilling stress

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Abstract: Introduction: Many plants of tropical and subtropical origin are severely damaged when exposed to chilling temperatures. Arabidopsis thaliana is chilling tolerant, but there are some mutants of this plant with chilling sensitive phenotype. These mutants can be classified in four groups based on visible phenotypes. Class 1 of the mutants have more severe of chlorotic and wilt phenotypes and die after 3 days of exposure to chilling temperatures. Plants are used the enzymatic antioxidant mechanism (such as SODs) as a defense system exposed to low temperatures. Cu-Zn SOD2 (CSD2) has the chloroplast location. To determine whether the expression of CSD2 would increase superoxide-scavenging capacity and thereby improve the survival rate of chilling sensitive (chs) mutants of Arabidopsis, four chs mutants (chs1-1, chs1-2, chs2-1 and chs2-2) and WT plants were grown under low (chilling at 13°C and cold at 4°C) and normal growth (23°C) temperatures. Method: This study was done in the master's thesis format in the Kharazmi University of Tehran, Iran. After the 4th week, M2 populations of chs and WT Arabidopsis plants (ecotype Colombia) were transferred to stress conditions for 1 week. CSD2 gene expression was assessed using semi-quantitative RT PCR method. Results: The expression of CSD2 were not detected during chilling and cold stress treatments, while the WT plants showed the expression of CSD2 under chilling and cold stresses. Conclusions: The lack of expression of CSD2 gene in chs mutants grown at chilling temperature would support the hypothesis that chloroplasts might be damaged due to the mutation, when they are chilled.

Keywords: Arabidopsis thaliana, chilling stress, Cu/Zn SOD, chs mutants.
Title:
Cloning and expression of Full-length form of Staphylococcal Protein A (SpA) in E. coli

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Abstract:
Introduction: Staphylococcal protein A (SpA) plays an important role in the Staphylococcus aureus pathogenesis. The recombinant form of SpA is used extensively in immunology and other biological research. The immobilization of SpA protein onto solid support is frequently used as a consistent and reliable method to purify total IgG from crude protein mixtures that include serum and ascitic fluid. It is also used in the detection of antibodies when coupled with one of the above markers. Method: In this study, a full-length form of SpA was cloned and expressed in Escherichia coli and confirmed by Western blot assay. Results: The results revealed that SpA was expressed and secreted into the medium. For confirmation, Western blot assay was performed using IgG anti goat HRP-conjugated antibodies and the result indicated that the protein was expected one. Conclusions: Expression and Secretion of proteins by E. coli into the growth medium is advantageous over intracellular expression. These benefits include lower production costs, simple downstream processing, and stability of expressed proteins and N-terminal authenticity of the expressed peptide.

Keywords: E. coli; Expression; Cloning; Full-length SpA.
Title:
Evaluation of biological activity in recombinant human granulocyte colony stimulating factor

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Abstract:
The biological activity is one of the important issues in quality control of recombinant protein drugs. Biological activity indicates the potential of product in biological effect induction. For bioassay evaluating, different methods are based on animal using, cell culture, biochemical and immunological techniques. However, this method is accepted when is accompanied to the quantitative measurement. In this study, the bioassay of recombinant human granulocyte colony stimulating factor (rh-G-CSF) produced in E.coli was evaluated based on cell culture, in comparative to Filgrastim. A proliferation assay using the NFS-60 cell line was used to assay biological activity of G-CSF. Samples to be examined and standard (Filgrastim) were serially diluted 1:2 in RPMI medium (10 serial dilution). After incubation of the cultures in 37 °C, 24 h, survival/or proliferating cells are detected at the end of assay by their ability to cleave the tetrazolium salt MTT and produced a colored product that is read in 497 nm. The results suggest that in the studied G-CSF concentration range, the cell proliferation is followed by sigmoid model. The biological activity of produced protein is estimated $0.9 \times 10^8$ IU/mg in comparative to filgrastim ($1 \times 10^8$ IU/mg) and according to the pharmacopoeia standards criteria.

Keywords: G-CSF, biological activity, cell culture
Title: Evaluation Mannose-binding lectin gene and promoter polymorphism in renal transplant recipients

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Abstract: Introduction: The Aim of present study was to determine the distribution of the alleles of mannose-binding lectin gene and promoter variants in renal transplant recipients and seek correlation between these variants and diseases that cause renal dysfunction. Method: One hundred and thirteen renal recipients samples were compared with 120 normal controls from Azarbaijan population of Iran. Blood samples were obtained from renal recipients who received a kidney between March 2004 and July 2005. Mannose-binding lectin genotypes were investigated by polymerase chain reaction and restriction fragment length polymorphism. Results: Allelic and genotypic frequency of the polymorphism at position -550, -221, +4 and at codon 52, 54 and 57 did not show statistical differences between recipients and controls (P >0.05) but significant frequency of allele B (codon 54) (P=0.02) and LX haplotype (P=0.002) of promoter was observed in this patients. Conclusions: Our findings provide evidence that presence of different alleles and haplotypes that cause low concentration of mannose-binding lectin in serum is a risk factor for susceptibility to renal infections that cause renal dysfunction.

Keywords: Polymorphism, Renal transplant, MBL, PCR.
Title:
Synthesis and in vitro evaluation of drug-loaded nanoparticles as new drug delivery system for colon cancer

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Abstract:
Introduction: Mainly colon targeted delivery system and ideal targeted drug delivery system is the one which delivers the drug only to its sites of action and not to the non-targeted organs or tissues. Nano particles hold tremendous potential as effective drug delivery system. Beta Lactoglobulin (BLG) is the main whey protein component. Because of the physicochemical properties of this protein such as stability in low pH and presence of protease in stomach and its ability for binding to hydrophobic ligands, BLG is suitable carrier for transportation of new designed Pt(II) complex (bipyridin ethyl dithiocarbamate Pt(II) nitrate), as an anticancer drug, against colon cancer.

Method: The effect of the pH on the size of Nano particles and stability of them were studied using dynamic light scattering (DLS) and scanning electron microscopic (SEM) techniques. Also, dialysis studies were used to examine drug release from Nano particles in the simulated gastrointestinal conditions.

Results: Results of DLS and SEM show that in pH< pl, because of electrostatic and formation solution complex, Nano particles with 200-250 nm in size and high colloidal stability were obtained. Drug release profile in simulated GI conditions demonstrated the stability of BLG and secondary coating in acidic conditions and its release at pH 7.

Conclusions: Our results suggest that this new drug delivery system for anticancer drugs will be able to deliver the anti-cancer drugs and provides a potential to enhance anti-tumor efficacy with low systemic toxicity in the treatment of cancers, especially for colon cancer.

Keywords: BLG, Pt (II) complex, Nano particle, DLS
Title:
Mannose-Binding Lectin Gene and Promoter Polymorphism and Susceptibility to Renal Dysfunction in Systemic Lupus Erythematosus

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Abstract:
Introduction: Systemic Lupus Erythematosus (SLE) is a prototypical autoimmune disease characterized by the production of autoantibodies and the deposition of immune complexes in effected end organs. Both genetic and environmental factors appear to contribute to the development of systemic lupus erythematosus. One of the genetic factor effective in case is Mannose-Binding Lectin (MBL) gene. The aim of present study is to was determine the distribution of the alleles A, B, C, D and H, L, P, Q variants and Hy, Ly, Lx haplotype in Lupus patients had renal dysfunction in compare with normal control. Method: Twelve SLE patients with sever renal failure were compared with thirty normal control from Azarbaijan population of Iran. Frequency of alleles and genotypes were investigated by polymerase chain reaction and restriction fragment length polymorphism. Twelve SLE patients with sever renal failure were compared with thirty normal control from Azarbaijan population of Iran. Frequency of alleles and genotypes were investigated by polymerase chain reaction and restriction fragment length polymorphism. Results: Allelic and genotypic frequency of the polymorphism at position-550, +4 and at codon52,54 and 57 did not show statistical differences between SLE patient and controls but frequency of Lx haplotype was observed in patients with SLE and renal failure (p=0.0518). Allelic and genotypic frequency of the polymorphism at position-550, +4 and at codon52,54 and 57 did not show statistical differences between SLE patient and controls but frequency of Lx haplotype was observed in patients with SLE and renal failure (p=0.0518). Conclusions: Present findings showed that presence of Lx haplotype that cause low concentration of MBL in serum can be a risk factor for severity of systemic lupus erythematosus and susceptibility to renal dysfunction. Present findings showed that presence of Lx haplotype that cause low concentration of MBL in serum can be a risk factor for severity of systemic lupus erythematosus and susceptibility to renal dysfunction.

Keywords: Polymorphism, MBL, PCR, SLE, RFLP.
Title: Study of miRNA presence in the plasma fractions of gastric cancer patients

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Abstract: Introduction: Current diagnostic tools lack adequate efficiency and sensitivity to detect the disease of gastric cancer in the early stages. So development of a novel diagnostic test which is able to detect cancer in early stages will be crucial and inevitable. In this study, we aimed to develop a strategy to fractionate plasma of gastric cancer patients and investigate the levels of selected miRNAs in those fractions compare to the normal samples. Method: In this controlled study, we investigated the panel of seven miRNAs in different fractions of plasma from three gastric cancer patients and healthy donors using quantitative RT-PCR. To set up the experiment, we recruited several techniques i.e. cell culture, RNA extraction, in vitro transcription, primer designing, and particle size measurements. Results: We could detect all miRNAs of our panel (let-7a, miR-20a, miR-21, miR-27a, miR-106a and miR-106b) in all plasma fractions from patient and normal samples. Expression analysis showed that levels of some miRNAs (miR-21, miR-27a, miR-106a and miR-106b) tend to be different in cancer and normal samples and each plasma fraction can have independent correlation with the cancer condition. Conclusions: The ultimate goal of this research is to find a robust and reproducible miRNA changes in cancer compare to normal plasma and use it as a biomarker for early diagnosis of gastric cancer patients. Our result provides a new opening in the search for miRNA biomarkers in plasma. In the future, larger number of samples should be employed and better methods can be applied for more accurate detection of miRNA level differences. Hopefully, further experiments will result in finding the miRNA biomarker of gastric cancer.

Keywords: microRNA, Cancer, Early diagnosis, Blood Plasma
Optimized Expression and Purification of Anabeana variabilis phenylalanine ammonia lyase in E. coli

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Abstract:
Introduction: There is an increasing interest in enzyme replacement therapy of metabolic diseases. Phenylketonuria has already known to be caused by phenylalanine hydroxylase (PAH) deficiency. Some attempts have been done to use recombinant PAHs, but the need of PAH to cofactor necessitates the use of alternate enzyme, phenylalanine ammonia lyase (PAL). Bacterial, fungal and plant PALs transform L-Phe to the much harmless metabolite trans-cinnamic acid. Anabeana variabilis PAL (AvPAL) has been recently introduced as a good candidate in clinical trials of phenylketonuria enzyme replacement therapy. In this study we used the dT7 promoter expression system to produce recombinant AvPAL in E. coli. The optimum conditions to obtain active enzyme was explored. Method: AvPAL gene was cloned into the pET expression vector and the protein expression was induced by IPTG. The presence of AvPAL protein was assessed by SDS-PAGE gel electrophoresis, in different conditions: time of induction, inducer concentration, temperature and culture media. The activity of the expressed AvPAL was assayed by uv-vis spectrophotometry. Results: Expression of AvPAL by T7 promoter system resulted in a high level of recombinant protein which appeared as a dense band in SDS-PAGE analysis of induced cultures. Optimization of condition for soluble active protein production revealed that the highest yield was obtained by induction with 0.2 mM of IPTG for 3 hours at 30°C. Conclusions: The results of our study demonstrate that the recombinant expression of PAL in E. coli by T7 expression system can be used for large scale production of recombinant PAL.

Keywords: Phenylketonuria, Phenylalanine ammonia lyase (PAL)
Title: Humanized single chain antibody humMR1, an antibody targeting EGFRvIII with great specificity

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Abstract: Introduction: Most of antibodies produced against EGFRvIII have the cross reactivity to wild-type EGFR as well. This drawback may limit their application in cancer therapy, since wild-type EGFR exists in nearly is found nearly in all human tissues. Considering the immunogenicity of non-human antibodies for human we decided to produce a humanized antibody to specifically target EGFRvIII without damaging normal tissues. This antibody was produced in single chain format.

Method: The protein sequence of MR1, a murine single chain antibody targeting EGFRvIII, was used to design humanized single chain antibody. Moreover, two tryptophan residues in CDR2 and CDR3 of heavy chain were converted to phenylalanine residues in order to eliminate the cross-reactivity of resultant antibody to wild-type EGFR. After designing the protein sequence of humanized antibody, the pertinent nucleotide sequence was determined, codon optimized, chemically synthesized and expressed in BL21 bacterial cells.

Results: the resultant humanized antibody, called humMR1, was able to recognized EGFRvIII specific synthetic peptide as lysate of HC2 cells, a cell line expressing EGFRvIII at high levels. This antibody also faintly recognized wild-type EGFR.

Conclusions: Alteration of the two tryptophan residues in CDR2 and CDR3 of heavy chain to phenylalanine residues improved the specificity of antibody toward EGFRvIII, but did not fully eliminate its cross reactivity to EGFR.

Keywords: EGFRvIII, humanization, single chain antibody, specificity
Title:
The study of seeding effects on fibrillation of Bovine Serum Albumin

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Abstract:
Introduction: A number of factors have been identified in increasing the kinetics of conformational change in amyloid forming. In this study, the evaluation of fibrillation process of BSA with and without seeds was done. Method: The samples were incubated at 70°C in a water bath, for 48 h, and a pH 3. To define the seeding conditions, aliquots of solutions of preformed BSA fibrils at pH 3 and 70 °C, were added to fresh solutions of the protein, and the kinetics of fibril formation were followed by means of thioflavin-T, a dye whose fluorescence increases significantly upon binding to amyloid fibrils. Results: In the absence of added fibrils, BSA exhibits lower fluorescence intensity under the chosen conditions after the certain time. By addition of aliquots containing preformed fibrils was found to result in a shortening of the lag phase indicative of seeding. When the percent of added fibrils become more (v/v), the increase in thioflavin-T fluorescence showed no lag phase and the maximum fluorescence intensity was reached after only 20 hours. Conclusions: This effect shows that the added seeds act as catalytic sites that induce conformational changes in the protein and allow the system to bypass the slow nucleation phase and reach the growth phase much faster and earlier.

Keywords: Seeding, BSA, Fibrillation, Fluorescence
Title:
mRNA display: applications in biotechnology and protein engineering

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Abstract:
Introduction: Protein engineering is one of the most intriguing disciplines in biotechnology. Display systems are powerful tools for protein engineering. Different cell-based protein display methods including phage display and cell surface display systems have been widely used for selection and evolution of proteins in vivo. However, they suffer from a relatively limited library size due to the restriction of cell transformation efficiency. So, cell-free protein synthesis (CFPS) display methods including mRNA display have been recently applied for protein engineering. In mRNA display, mRNAs are first translated and then covalently bonded to the protein they encode, using puromycin as an adaptor molecule. All the complexes are then exposed to the target molecules. Those peptides having the most affinity for the target molecule are selected and their related mRNAs are amplified. Successful examples of high-affinity, specific target-binding molecules selected by mRNA display method include peptides, antibodies, enzymes and engineered scaffolds. Conclusion: Techniques for performing mRNA display are now well established and enable facile synthesis and selection of mRNA-protein fusion complexes. mRNA display enables scientists to generate high specific proteins against various target molecules and is a promising tool in the field of protein engineering.

Keywords: Biotechnology, Cell-free protein synthesis, mRNA display
Title:
No association between Interleukin 27 promoter gene polymorphism and risk of type1 diabetes in the Azerbaijan, Northwest Iran

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Abstract:
Introduction: Interleukin 27 (IL-27) is a newly discovered cytokine, consists of two subunits, the Epstein-Barr virus-induced gene 3 (EBI3) and p28. It can promote both anti- and pro-inflammatory immune responses, also TH1 differentiation. IL-27 has been linked to the activation of CD8+ T cells and promotion humoral responses. Therefore, it has been proposed that IL-27 plays a potential role in autoimmune diabetes. However, data regarding to the role of IL-27 in autoimmune diabetes are scarce. Thus, the aim of this study was to investigate p28 gene -964 A>G polymorphism in Type 1 diabetes mellitus (T1D) compared to healthy control group. Method: DNA was extracted from blood samples of 156 T1D patients and 179 sex, age and ethnically matched healthy controls. Flanking region of -964 position of the IL-27 p28 subunit encompassing 468 bp nucleotides was amplified by PCR and analyzed by restriction fragment length polymorphism (PCR-RFLP), and separated by agarose gel electrophoresis. Results: Our results demonstrated that the alleles and genotype frequencies of the -964 A>G polymorphism of IL-27 p28 in the T1D patients were not significantly different from those of the healthy control group (P = 0.185 and P = 0.355; respectively). Conclusions: Our findings suggest the -964 A>G polymorphism of the IL-27 gene promoter region is not associated with susceptibility to T1D in the Azeri population from Northwest of Iran.

Keywords: interleukin-27 gene, Type 1 diabetes, single nucleotide polymorphism, PCR-RFLP.
Title:
Studies of the Relationship Between Structure and Antioxidant Activity in interesting systems, including tyrosol, hydroxytyrosol derivatives Indicated by quantum chemical calculations

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Abstract:
In nature, hydroxytyrosol and tyrosol are found in olive leaf which are for medical purposes, with Immune stimulant and antibiotic properties and can be useful drugs for the treatment of neurodegenerative diseases such as Alzheimer's. The radical scavenging potential of phenolic compounds occurring in tyrosol and hydroxy tyrosol, tyrosol and hydroxy tyrosol are sensitive to oxidation and are hydrophilic. Hydroxytyrosol (3,4-dihydroxyphenylethanol; DOPET) is a phytochemical with antioxidant properties. Hydroxytyrosol and derivatives are believed to be one of the most powerful antioxidants. In order to establish the possible structure-antioxidant activity relationship, hydroxytyrosol and tyrosol and hydroxytyrosol acetate and two derivatives of newly designed hydroxytyrosol by some physicochemical parameters (heat formation of the neutral, radical, orbitals energies, Clog P, BDE) were evaluated by means of quantum chemical calculations. The bond dissociation enthalpy (BDE) of phenolic hydroxyl groups was calculated as descriptors to predict the H-atom-donating abilities of antioxidants. Catechol derivatives had the lowest BDE values (77.7-80.1 kcal/mol). Considering the results from the calculated descriptors, the derivatives with hydroxyl group substituents in position number five of the ring can be classified as having a higher antioxidant activity. The results lead to the conclusion that hydroxytyrosol derivatives which have been designed are the most active compounds with higher antioxidant potency. This work may be useful to clarify the radical scavenging mechanism of antioxidants and to design novel antioxidants.

Keywords: Antioxidant; hydroxytyrosol; Bond dissociation enthalpy; BDE; Free radical;
Title:
Bioactive peptides produced by autolysis of ficin extracted from fig (Ficus carica cv. Sabz)

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Abstract:
Fig fruit contains 1.5% proteins which approximately 90% of them are ficin. Naturally ficin undergoes autolysis. Thus, the study of biological activity of peptides derived from the breakdown of fig fruit proteins, especially the antioxidant properties of ficin peptides, is necessary. Ficin of Iranian fig indicates the autolysis process. This phenomenon is different between ficin isoforms due to their different structure. In this experiment, the produced peptides from 42-hours autolysis were fractionated with 10, 5 and 3 kDa ultrafiltration membranes. Their antioxidant activities were determined using a Trolox equivalent antioxidant capacity (TEAC). The results showed that bioactive peptides produced from ficin autolysis have high antioxidant activities. Peptides with molecular weight of 5-10 kD showed the highest antioxidant activity. Fig can be introduced as a functional fruit that has nutraceutical properties due to the presence of autolysis ficin to make bioactive peptides which are affected in the treatment and prevention of numerous diseases.

Keywords: Antioxidant activity, Autolysis, Ficin, Fig, peptides.
Title:
Cloning and Expression of Aspergillus niger Endoinulinase gene in E.Coli

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Abstract:
Endoinulinase is a 2,1-β-D-fructan fructanohydrolase from glycosyl hydrolase family 32. This enzyme hydrolyses the β(2→1) bonds of inulin and fructan chains. Inulin is a polymer of fructose stored in the roots of plants such as chicory, dahlia and Jerusalem Artichoke. Endoinulinase can be used for production of inulooligosaccharide from inulin. Inulooligosaccharide has prebiotic properties and using it in the diet leads to reducing of colon cancer catching risk. Endoinulinase furthermore can be used together with Exoinulinase for production of High Fructose Syrups (HFSs) and ethanol from inulin. Using fructose in the diet instead of sucrose, can enhance absorption of calcium and iron. Fructose is better tolerated by diabetic patient and enhances removal of ethanol from the blood of alcoholics. In this project endoinulinase gene of A. niger PTCC5012 was amplified by using PCR. This gene consisted of 1485bp encoding the protein of 494 amino acids. The mature protein contained two cys residues and five potential N-linked glycosilation site. The PCR product was digested with Hind III and SacI enzymes and was cloned into pQE30 vector which previously digested with the same enzymes. E.coli top10 was used as expression host. Expression of endoinulinase was confirmed by SDS-PAGE and enzyme assay.

Keywords: Endoinulinase, A.niger
Title: Effects of osmolytes on the Protein stability: Molecular dynamics simulation

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Abstract: The presences of osmolytes in aqueous solution have significant effects on providing essential interactions for the unique three-dimensional conformation of native proteins. Osmolytes such as trehalose or sucrose have been known to protect proteins against loss of activity and prevent the partial or even total degradation of biomolecules. Despite various experimental and theoretical works, the detailed molecular mechanism at the origin of polyols protective ability still not well understood. MD simulations can provide valuable information about the different stages of peptide–solvent interactions.

Methods: All simulations were performed using the gromacs software package, version 4.5.4. The Gromos96 53a6 parameter set was used as force field. The starting conformation of the peptide was obtained from the protein databank (PDB) structure 1LCI. The peptide was solvated with SPC water, a mixture of sucrose, a mixture of trehalose.

Results: Our results showed the total number of hydrogen bonds between the protein and solvents over the course of the MD simulations is not significant. Nevertheless there is main difference in water and osmolyte direct interaction with protein. The water molecules mainly interact with protein side chains while osmolytes are more involved with protein backbone. Also, we have identified variations in the secondary structure of the protein during the simulations. In pure water some part of alpha helical conformation of protein is deteriorates with time whereas in sucrose solution protein secondary structure is maintained throughout the 100 ns duration of simulations.

Conclusions: In conclusion, according to the results presented in this study it has been suggested that osmolyte with effect on special part of protein and make favorable environment, can prevent protein thermal unfolding.

Keywords: Osmolytes, Protein stability, Protein–Osmolyte interactions, Molecular Dynamics Simulation
Title: Hydrolysis Method Of Date Palm Leaf To Produce Bioethanol

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Abstract: High oil prices with concerns about carbon di oxide emissions have resulted renewed interests in renewable energy. Bioethanol has been recognized and used as a potential alternative to petroleum-derived transportation fuels. In this study we used acid hydrolysis in different situation (concentrations, times and temperatures). In this study we used of 4 different concentrations, 3 of different temperatures and 4 of different times to pretreatment. Finally we used of Lane-Eynons method to measurement of sugar percentage. According to results, the highest percentage of sugar (50%) obtained at concentration of 3.12% HCL at 70 centigrade and time was 24 hours. Our results exhibit, Palm wastes are very great for bioethanol production and HCL is one of the beneficial acids in optimization and separating of sugar.

Keywords: Date Palm Leaf, HCL, Bioethanol
Title: Estimation of the Interactions between the Factors Affecting Microbial Soil Strengthening by Ureolytic Sporosarcina pasteurii

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Abstract: Introduction: Microbially-Induced Calcite Precipitation (MICP) is a novel soil improvement technology. The bacterial ureolysis precipitates carbonate that improve soil mechanical characteristics. Various advantages of this technique necessitate its optimization for field practices. Present study provides a precise insight into the interactions between the major controlling factors of this process. Method: An orthogonal array (L16) was designed using Taguchi methodology representing the experimental conditions of the trials. Soil type, curing time, inoculum size, nutrient solution concentration, and bacterial cell density were studied in four levels. Soil specimens were treated with Sporosarcina pasteurii PTCC 1645 and nutrients and unconfined compressive strength (UCS) test was performed after desirable curing time. The results were analyzed using Qualitek-4 Software to quantify the factors interactions. Results: All the trials showed measurable increase in strength ranging from 4716.86 to 104.83 kPa. Severity index (SI) was computed for possible interactions between pairs of factors. The highest SI were calculated for pairs of nutrient concentration × inoculum size (74.02%), nutrient concentration × curing time (63.99%), and inoculum size × curing time (52.07%). The least value (19.6%) was achieved for bacterial concentration × inoculum size pair. The interaction between soil type and biomass concentration with the other parameters were below 50 and 40%, respectively. Conclusions: Bacterial treatment proved promising for soil strengthening. The bacterial cell density and soil type are more independent factors than the others. This outcome along with the relatively high interactions between inoculation size, nutrient concentration, and curing time should be considered for follow up optimization experiments.

Keywords: Interaction, Factors, Microbial Soil Strengthening, Taguchi method, Severity Index.
Title:
The study of cadmium biosorption by an indigenous cyanobacterial strain Nostoc spp. isolated from contaminated soils

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Abstract:
Introduction: Cadmium is an important environmental pollutant and very toxic heavy metal. Excess cadmium in the organisms can damage DNA sequencing and may cause genetic changes and cancer. Biosorption is a technique that can be used for the removal of metal pollutants from waters. Cyanobacteria are the largest and most diverse group of photosynthetic prokaryotes that represent the best biological treatment for wastewater because they increase O2 content of waters via photosynthesis and sorption of some heavy metals contaminated waters such as cadmium.

Methods: Biosorption of Cd (II) ions from aqueous solution using lyophilized cells of cyanobacterium Nostoc spp. was studied under various experimental conditions. The effect of parameters like pH, contact time and initial metal concentration was investigated in batch experiments. The mechanism of Cd (II) adsorption on Nostoc powder was defined by applying pseudo first- and second-order rate equations. Langmuir and Freundlich adsorption isotherms were employed to understand the nature of sorption.

Results: The optimum conditions of Cd biosorption were as following: 5mg of dried biomass, 150 mg/l Cd (initial concentration), and pH 6, 30°C, and 30 min (contact time). The result obtained from this study demonstrated that Langmuir isotherm had a higher correlation coefficient than the Freundlich isotherm ($R^2=0.908$) and maximum uptake capacity of Cd (II) was estimated to be 333.33 mg/g.

Conclusions: All the studied parameters indicated that this native cyanobacterial strain with high biosorption capacity of Cd toxic metal can eventually be used as a source for a novel approach in using biosystems to remediate contaminants from environmental ecosystems.

Keywords: Cyanobacteria, Nostoc spp, Biosorption, Cadmium, Heavy Metal
Title: Mannose-binding lectin gene and promoter polymorphism in visceral leishmaniasis caused by Leishmania infantum

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Abstract: Introduction: Visceral leishmaniasis (VL) is an infectious disease caused by various species of Leishmania. *Leishmania infantum* is known to be associated with VL in Iran. Different factors are considered as risk factors for VL. The aim of this study is to determine the frequency of mannose-binding lectin (MBL) gene and promoter variants and seeking the correlation between MBL alleles and developing of VL.

Method: Blood samples of 58 patients with confirmed VL were compared with blood samples of 120 normal controls from Azarbaijan population of Iran. MBL genotypes were investigated using polymerase chain reaction and restriction fragment length polymorphism techniques.

Results: Frequency of alleles with high MBL concentration was higher in VL patients than in controls (P = 0.03), but no differences were demonstrated when other alleles were compared between healthy individuals and patients.

Conclusion: Low-expression MBL genotype can be associated with protection against VL cause by *Leishmania infantum*. In addition, wild type alleles along with high MBL level can be considered as a risk factor for confirming visceral leishmaniasis.

Keywords: Leishmania infantum, Visceral leishmaniasis, Mannose-binding lectin, Polymerase chain reaction, Restriction fragment length polymorphism
Title:
Effect of bulk velocity, reactive concentration and device configuration on microfluidic biomolecule-capturing devices with reactive surfaces

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Abstract:
Microfluidics refers to devices and methods for controlling and manipulating fluid flows with length scales less than a millimeter. These technologies have tremendous potential for creating portable health diagnostic devices due to advantages in size, volume requirement, and time to analysis. A two-dimensional computational model is employed to include the mass transport (convection and diffusion) in the bulk flow and reaction on reactive surfaces. Since the bimolecular reactions take place at the surfaces, finding the optimal device configuration to capture as many biomolecules as possible on the surface is a crucial step in the design process. In this study, the effect of the reactive surface configuration on the device performance will be investigated. Then, the influence of the bulk velocity and the concentration of the reactive surfaces will be examined. The determination of the optimal configuration is very complicated due to the large number of the parameters governing the physics of the problem. Packed-bed designs (including circular micropillars with different configurations) are proved to enhance the reaction performance. This is a direct result of reducing the diffusion path and using convection in biomolecule transport. Hence, to increase the reactive surface facing the flow, the most distributed pattern of circular pillars should be taken into consideration. Two designs of five and six circular pillars, left and right-sided, are investigated. Capture efficiency is calculated for both five and six circular reactive surfaces. The numerical model presented here also allows the thorough examination of the effect of design parameters on the device efficiency. It is shown that adding to the number of reactive plates often increases average surface concentration of the device.

Keywords: Microfluidic device, biomolecule, convection, diffusion
Title:
Chloroperoxidase nano-artificial enzyme designed base on cystein-TTAB-Fe-Protoporphyrin complex

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Abstract:
A nano-artificial enzyme was designed base on the principle of biomimetic chemistry in acidic condition at pH 3. The Fe- Protoporphyrin reacted to form heme-like center. The Fe ions show catalytic activity in presence of Cys, which provide the axial bond on Iron. Tetradecyltrimethylammonium bromide (TTAB), a Cationic surfactant, is used to mimic the hydrophobic packet of chloroperoxidase enzyme. In this research the new designed artificial enzyme performed in micellar solution. Catalytic activity of nano enzyme is 25% compare to the native chloroperoxidase.

Keywords: Protoporphyrin, Artificial enzyme, Chloroperoxidase, Cystein, TTAB
Title: Determination of antioxidant activity, phenol and flavonoid content of Stachys Lavanduliflia

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Abstract: Introduction: Natural antioxidants like fruits, vegetables and medicinal plant play an important role in human healthy via their ability to inhibit free radicals. Oxidative stresses originated from free radicals are cause of many diseases such as a variety of cancers, heart disease Alzheimer and Parkinson diseases. We studied here the antioxidant effects of methanol and ethyl acetate extracts of Stachys Lavanduliflia as natural antioxidants. Materials and methods: Methanol extracts (by three methods: maceration, ultrasonic, soxhlet) as well as ethyl acetate extract (by maceration method) was prepared. Total phenol compounds, was determined by the Folin Ciocalteu method and the total flavonoid content, by AlCl₃ method. Fe²⁺ chelating ability and 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) were used to evaluate antioxidant activities. Results: The highest content of phenol and flavonoid was obtained in ethyl acetate extract. Themaximum IC₅₀ for DPPH radical-scavenging activity was obtained in methanolic extract (soxhlet). Themaximum Fe²⁺ chelating ability was obtained extract of ethyl acetate (64.5%). Results showed the antioxidant activity related to total phenol and total flavonoid content (r² = 0.76 and r² = 0.43 respectively). Conclusion: Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities. Studies have shown that increasing levelsof Phenols and flavonoids in the diet could decrease certain human diseases.

Keywords: Stachys Lavanduliflia, antioxidant activity, Methanol extract and ethyl acetate extract
Title: Differential protein expression analysis between grade III and grade IV Glioma by a 2D-DIGE technique

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Abstract: Oncoproteomics is increasingly employed in both neurological and oncological biology research to provide insight into molecular basis of disease but rarely achieve coherence. Brain Glioma are the most common primary human brain malignant tumors and diffuse Gliomas are highly invasive, heterogeneous and respond poorly to treatments. Differential protein expression between various pathological grades of malignant Gliomas has been shown in studies of Glioma proteomics. For comparative proteomic analysis, samples selected from high grade (III and IV) Glioma and tumor-sparse proteins were extracted. We separated proteins by 2D-DIGE technique and the spots were then analyzed and compared using specific software, after providing 3D images of spots alteration. Results revealing approximately 800 protein spots by 2D-PAGE, including spots increased and decreased expression. Comparison of grade III and grade IV Glioma revealed 448 differentially expressed and statistically significant (p < 0.05) protein spots. Proteins play active roles in biology and metabolic pathways through their effect on tumor cell proliferation, tumor differentiation, tumor malignancy, metastasis and cell death.

Keywords: Proteomics, Glioma, 2D-DIGE and Brain Tumor
Title:
Characterization of the structural changes of HSA upon binding with Propranolol, Alprazolam, Quercetin and Taxifolin by means of Molecular Dynamic Simulation

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Abstract:
Introduction: Human Serum Albumine (HSA) binds with drugs under normal physiological conditions. Drug binding with HSA can change the structure of protein. The structural changes within HSA due to its interaction with drugs could be considered as a drug side effect and it may affect the protein functions. We used molecular dynamics (MD) simulations to clarify possible structural changes of HSA brought about the binding of drugs. The structural analysis of unliganded HSA and HSA complexes with propranolol, alprazolam, quercetin and taxifolin are reported.

Method: All calculations were carried out using GROMACS 4.0.5 package and GROMOS 96 force field.

Results: The Cα RMSD values of protein were reduced and reached to equilibrium after ligand binding. Th Rg plot revealed that HSA folded upon binding with taxifolin, counter propranolol and quercetin caused HSA unfolded. After ligand binding, the flexibility of some residues that were not in direct contact with ligand molecules increased significantly, indicating an increase of entropy in this regions. The residue - residue contact map showed the tertiary structure of HSA changed after ligand binding. Thus, the secondary structure changes of HSA for complexes were monitored. Results displayed that the helix content of HSA declined, specially with quercetin.

Conclusions: In this study, we shows that MD simulations can be used to determine structural differences of HSA upon ligand binding. Drug binding affect the HSA structure and could alter the physiochemical properties of HSA. This effect can be considered as a drug side effect.

Keywords: Human serum albumine, Molecular dynamic simulation, Conformational change, Ligand binding
Title: Humanisation of anti-EGFR and its expression in E. coli

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Abstract: Introduction: Epidermal Growth Factor Receptor is involved in a number of cancers and has been considered as a good target for cancer therapy. Cetuximab is a chimeric anti-EGFR monoclonal antibody used for treatment of colorectal cancer and head and neck cancers. However, immunogenicity problems pertaining to mouse-derived monoclonal antibodies and large size of full-length antibodies are two major obstacles in cancer immunotherapy. Humanized single chain antibodies can overcome these difficulties and are considered as proper alternatives in cancer immunotherapy. The aim of our study was to produce a humanized scFv antibody from chimeric Cetuximab for potential use in diagnosis and treatment of EGFR expressing solid tumors. Method: A gemline-based approach was used to design a humanized scFv gene fragment. While retaining the murine key residues, the CDRs were grafted onto the closest human frameworks. The designed construct encoding heavy and light chains were joined together using a linker peptide and expressed in Escherichia coli to obtain recombinant scFv. The expression level analysis was conducted by SDS-PAGE. Result: by similarity analyses it was revealed that human germline genes IGHV3-33*04 and IGKV3-15*01 had the highest homology to their murine counterparts. SDS-PAGE analysis showed a high expression level in E. coli. Conclusion: The results of this study indicated that E. coli expression system capable of producing high level of recombinant scFv and could be used for large scale production of single chain antibodies.

Keywords: Single-chain antibody variable fragment, Antibody humanization, Anti-EGFR
Title: NMR-Monitored Hydrogen Exchange Study on the Conformational Stability of RNase A: The effect of cationic gemini surfactants

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Abstract: Because of the many applications of surfactants in bioscience, drug delivery, and biotechnological processes, they are one of the most significant classes of applied ligands and their interaction with different proteins has been studied widely. The conformational stability of ribonuclease A (RNase A) has been measured at the per residue level by NMR-monitored hydrogen exchange in the absence and presence of cationic gemini surfactants. The hydrogen/deutrium exchange mechanism of RNase A has been found EXII in these conditions. We used gemini surfactants alkanediyl -α,ω-bis (Hydroxy ethyl methyl hexadecyl ammonium bromide) in this study. 1D NMR experiments showed gemini surfactants bind to DSS. 2D 1H-NMR spectroscopy shows that the conformation of RNase A is unaffected at acidic pH where this protein is positively charged, although hydrogen exchange results shows that the conformational stability of RNase A is slightly lowered at high molar ratios and acidic pH. At low molar ratios, the denaturation curves of RNase A in the presence of gemini surfactants were done by absorbance spectroscopy. These curves were analyzed on basis of a two-transition model. These gemini surfactants slightly activate and stabilize RNase A at low molar ratios. The gemini surfactant with the shorter spacer interacts more efficiently with RNase A than those with longer spacers. These gemini surfactants neither interact strongly with nor severely destabilize this well folded protein in physiological conditions and we advance that can serve as useful membrane mimetics for studying interactions between membrane components and positively charged proteins.

Keywords: Ribonuclease A, Hydrogen Exchange, Cationic gemini surfactants, Thermal denaturation
Title: Quantum mechanical study of antioxidative ability and antioxidative mechanism of chrysin in solution

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Abstract: Introduction: Chrysin is a biologically active natural molecule that present in many plants and belongs to flavonoids family. Flavonoids are the most important class of polyphenolic compounds, which in addition to their important biological roles in plant pigmentation, nitrogen fixation, and chemical defense possess anti-cancer, anti-inflammatory, antibacterial, antiviral, and anti-allergic properties that are a consequence of their antioxidant properties. Antioxidants are compounds that can prevent biological and chemical substances from oxidative damage by reactive oxygen species such as the hydroxyl radical, the superoxide radical, singlet oxygen and lipid peroxyl radicals. The objectives of this study are to investigate the antioxidant of chrysin including hydroxyl radical scavenging activity. Method: Quantum mechanical calculation were carried out using the Gaussian program series 2003 with DFT method at the B3LYP/6-311++G** level in gas and solvent phase by using PCM method. Results: The interaction between chrysin and hydroxyl radical has been simulated by using quantum mechanical calculations and B3LYP/ 6-311++G** method. The results of our calculations indicate that the oxidation of chrysin by hydroxyl radical is an exothermic reaction about 27 kcal/mol. Our calculation has been done in the gas and in solution phase. Conclusions: According to our calculated results, chrysin has tendency to donate an electron to active radicals, converting them into more stable non-reactive species and terminating the free-radical chain reaction. The molecular properties that we used to investigate a possible antioxidant mechanism of chrysin were the spin density distribution of the radical formed after the abstraction of the hydrogen atom.

Keywords: Chrysin, Antioxidant, QM calculation, Exothermic
Title: PLGA-based nanoparticles as cancer drug delivery systems

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Abstract: Background: Poly (lactic-co-glycolic acid) (PLGA) is one of the most efficiently developed biodegradable polymeric nanoparticles. It has been approved by the US FDA for the make use of drug delivery systems due to their controlled and sustained-release properties, less toxicity, and biocompatibility with tissue and cells. As mortality owing to cancer continues to rise, advances in nanotechnology have considerably become a successful approach for achieving efficient drug targeting to tumor tissues. Poly (lactic-co-glycolic acid) (PLGA) was synthesized by ring-opening polymerization of DL-lactide and glicolide. The bulk properties of these copolymers were characterized using 1H nuclear magnetic resonance spectroscopy, Gel permeation chromatography, Fourier transform infrared spectroscopy and Differential scanning calorimetry.

Conclusion: There is potential for use of these nanoparticles for biomedical application. PLGA has been the center of attention for developing drug-loaded nanoparticles for cancer therapy. In this review, the methods of preparation and characterization, various surface modifications, encapsulation of diverse anticancer drugs, active or passive tumor targeting and different release mechanisms of PLGA nanoparticles have been discussed. Increasing experience in the application of PLGA nanoparticles has provided promising future for use of these nanoparticles in cancer treatment, with higher efficacy and fewer side effects.

Keywords: Nanotechnology, Poly (lactic-co-glycolic acid) (PLGA), Drug delivery, Anticancer drugs
Title: fabrication of a Choline Biosensor Using Carbon nanotubes/Ionic Liquid/Prussian Blue Modified Glassy Carbon Electrode

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Abstract: Introduction: Choline is considered as a biomarker in the diagnosis of Alzheimer’s and Parkinson’s disease and prostate cancer. An amperometric biosensor for choline has been fabricated. Method: By composing multi-walled carbon nanotubes and the room temperature ionic liquid of 1-butyl-3-methylimidazolium hexafluorophosphate a Bucky gel was made. The prussian blue nanoparticles were then electrodeposited onto the modified electrode surface. The choline oxidase was subsequently immobilized by using a cross linking method. Results: Choline was detected in the concentration range from 0.5 µM to 175 µM with a detection limit of 0.5 µM at S/N=3. The modified electrode showed a sensitivity of 194.267 µA mM⁻¹ cm² toward choline. Conclusions: The sensor exhibited good electrocatalytic behavior towards the electro-oxidation of choline.

Keywords: Choline, Biosensor, Carbon nanotubes, Ionic liquid, Prussian blue
Title: A Study on the Effects of Some Polyphenolic Molecules on Aβ (25-35)

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder which has been a major public health problem with no cure or effective treatment. Although the cause of AD is not fully understood, studies indicate that aggregation of misfolded amyloid β peptide (Aβ) is the central event in the disease pathogenesis. The pathophysiological properties of Aβ can be studied through Aβ25-35 which is a special Aβ species retaining both physical and biological properties of intact Aβ. In order to prevent or reverse Aβ aggregation, diverse therapeutic strategies have been employed such as the application of small molecules. In the present work, the ability of some small, natural, and nontoxic polyphenolic molecules including resveratrol was investigated on preventing amyloid formation or disruption of preformed Aβ25-35 fibrils. Various techniques including fluorescence (ThT), Circular Dichroism (CD) spectroscopy, and Transmission Electron Microscopy (TEM) were applied to characterize the inhibitory effect of these small molecules. The inhibition of fibril formation and clearance of fibrillar structures of Aβ25-35 in deionized water were confirmed by the applied techniques in presence of polyphenolic molecules. Obtained data clearly demonstrated the inhibitory effect of these small polyphenolic molecules in the amyloidogenesis pathway of Aβ25-35. Development and application of such natural and nontoxic molecules with the ability to interfere with Aβ aggregation could offer great promises for efficient therapies of Alzheimer's disease.

Keywords: Alzheimer's disease, Amyloid beta peptide, Aggregation, Polyphenolic molecules, Aggregation inhibitors
**Title:**
Measurement of Aβ1-40 level in the peripheral blood of the Alzheimer’s patients

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**Abstract:**
**Introduction:** Alzheimer’s disease (AD) has been identified as a protein-misfolding disease, caused by accumulation of abnormally folded amyloid beta (Aβ) and tau proteins in the brain. Aβ1-40 and Aβ1-42 are the main components of amyloid plaques that are found in the brain of patients with AD. Identification of an AD-biomarker that can be derived from blood samples has been a goal of researchers for many years. Here, we have tried to determine the Aβ1-40 levels in the peripheral blood samples obtained from AD patients. Our preliminary results indicated that the levels of Aβ1-40 in the peripheral blood could be of considerable value in the differentiation of AD patient from normally aged individuals.

**Method:** We made use of a sandwich enzyme-linked immunosorbent assay (ELISA) to quantify blood serum Aβ1-40 level. Clinical diagnoses were carried out on the basis of the criteria of the NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders Association Stroke and Alzheimer’s Disease and Related Disorders Association). We analyzed 45 samples in three groups, i.e., 15 AD patients (aged 73.5±9.9), aged control (AC) group (10 individuals, aged 65.8±8.1) and young control (YC) group (20 individuals aged 28±3).

**Results:** The serum Aβ1-40 levels in the AD group were significantly lower than those in the other groups. Considering the relationship between Aβ1-40 level and age in AD group it become clear that Aβ1-40 level decreases with age in both females and males with AD.

**Conclusions:** These results are consistent with our hypothesis that low serum Aβ1-40 levels is an indicator of risk for AD and declines with onset and progression of Alzheimer and which might reflect the sequestration of Aβ1-40 in the senile plaques or the formation of semi-soluble oligomers.

**Keywords:** Keywords: Protein misfolding, Amyloid plaques, Biomarker, Amyloid beta.
Title:
Graft-versus-host disease in BALB/c mice after injection of human peripheral blood leukocytes

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Abstract:
Introduction: Bone marrow transplantation (BMT) has been known as the most effective treatment modality for a wide variety of hematologic, oncologic and metabolic disorders. A major obstacle to long-term success of BMT is its association with a serious side effect called acute graft-versus-host disease (GVHD). Despite using immunosuppressive drugs, the incidence of acute GVHD is high. The goal of present study is to investigate acute GVHD in BALB/c mice after the injection of human peripheral blood leukocytes.

Method: Immunosuppression was carried out by thymectomizing young mice and exposing to 8 Gy gamma ray. Human leukocytes were then injected and mice were studied after six days.

Result: Clinical signs of acute GVHD were seen in mice. These included humpback, curling hairs, dermatitis, diarrhea, hemorrhage, erythroderma and desquamation. Histological hyperplasia and infiltration of inflammatory cells were distinctive in spleen.

Conclusion: Initial manifestation of acute GVHD which was observed in mice and presence of reacting giant cells as well as hyperplasia showed the role of transferred leukocytes and interaction of cytokines in GVHD. Hence, we are probably able to inhibit GVHD by administering new anti-cytokine drugs.

Keywords: Immunosuppression, Graft-versus-host disease, Leukocytes, BALB/c mice
Title: Capacity of M13 phage particles in transgene delivery and expression in eukaryotic cells

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Abstract: Introduction: Gene therapy is one of the most exciting ways of genetic and hereditary diseases treatments. Of all many aspects of gene therapy process, delivering the transgene into the target cells is the critical one. Therefore, nowadays many gene delivery vehicles are considered to be manipulated. Beside, due to high stability in different conditions, low immunogenicity, simple genetic structure and production in large scale, bacteriophages are an appropriate alternative option for gene therapy and gene delivery vehicles. In this experiment M13 phage particles ability of internalization and expression of transgene is examined.

Material and methods: GFP gene sequence is cloned into pCMV-Script EX phagemid vector and after amplification and packaging of phage particles, they transfected to AGS cell line. Finally efficiency of internalization and expression of the GFP is examined by PCR and fluorescence microscopy.

Results: Low expression of GFP gene in AGS cells, indicates that M13 phage has no tropism to transfect eukaryotic cells.

Conclusion: Despite most of other gene delivery vehicles, bacteriophages have no tropism to eukaryotic cells, so coupling of different targeting moieties on the phage particles surface, might lead to efficient internalization to the target cells. This finding established application of bacteriophages as a unique class of gene carriers into eukaryotic cells.

Keywords: gene therapy, gene delivery, M13 phage.
Title:
Binding of oxali-palladium to β-lactoglobulin: A spectroscopic approach

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Abstract:
In recent years, medical chemistry has been faced with palladium (II) complexes with promising activity against tumor cell lines. Hence, spectroscopic and biological studies have been carried out in order to obtain information on structure-activity relationships for systems involving palladium (II) atoms. β-Lactoglobulin (β-LG), as a member of lipocalin family, according to its structure is known to bind a wide range of ligands. Also, numerous studies have been shown the benefits of β-LG as a carrier for drug delivery system. In the present work, the interaction of new synthesized oxali-palladium with β-LG was investigated in the aqueous solution with ambient temperatures (25 and 37 °C) by means of spectroscopic methods including fluorescence and circular dichroism (CD). The results of fluorescence measurements illustrated that the oxali-palladium quenches the intrinsic fluorescence intensity of β-LG with combined mechanism so that the static quenching is predominant. Using modified Stern-Volmer equation the number of binding sites was determined close to 2 at both temperatures of 25 and 37 °C. Also, thermodynamic parameters of ΔH° and ΔS° of this interaction were calculated, which revealed the hydrophobic force plays a major role in the binding process. CD measurements showed that the secondary structure of β-LG does not significant change by the interaction of oxali-palladium. We believe that these results are very helpful in pharmaceutical sciences, especially in drug delivery systems whereas can be open the door to new world of pharmacology.

Keywords: β-LG, Oxali-palladium, spectroscopy, binding force, quenching
Title: CpsD Protein, A Candidate Vaccine against Streptococcus iniae

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Abstract: Introduction: Streptococcus is one of the bacterial infections in fish especially rainbow trout which infects brain and nervous system of fish and is caused by streptococcal S. iniae bacteria. S. iniae is considered as a zoonotic pathogen and also a major cause of morbidity in many regions of the world. This bacterium is the most important pathogen of fish which incurs more than $100 million damages for fish farming annually. Polysaccharides capsule plays a critical and vital role in protecting pathogenic microbes against innate defense of the host during infection and will result in protection against phagocytosis. The streptococcal capsule aprons of an area (with an area of approximately 21 kb with 700 nucleotides) contain a group of multiple conserved genes (cpsA-E), which are responsible for polymerization and determination of length of capsule chain and placing it on the bacteria surface. Presenting a proper vector for designing vaccine against S. iniae for aquatic animals is the main aim of this study.

Method: In this study, cpsD gene was cut after being synthesized with Xbal and SacI enzymes and was purified from gel and then, it was cloned into the expression vector (pNZ8148). In the same direction, the new constructed vector was transformed to the bacterial expression E. coli strain BL21. To approve the expressed protein, Western Blot method is used.

Results: The cloning results of this gene were confirmed using molecular methods such as PCR. The protein was expressed and its validation stages are underway with antibody and Western Blot.

Conclusions: Cloning, sub-cloning and expression of cpsD gene are confirmed in the present study. Therefore, this recombinant protein can be used for production of a recombinant vaccine against Streptococcus iniae in future studies, after immunogenicity assay.

Keywords: cloning, Streptococcus, Streptococcus iniae, cpsD gene, pNZ8148
Title:
Identification and analysis of Dicer like proteins (Dicer-like) in poplar (Populus trichocarpa L.)

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Abstract:
Dicer or Dicer-like (DCL) proteins in gen silencing process has function and involved in the digestion of long double stranded RNAs with argonaute protein. Dicer-like (DCL) proteins one of the members of RNaseIII family and has key rolls in creation of small RNA. This protein cut the double RNA strand and convert that two RNA strand. The purpose of this research, study of Dicer-like (DCL) proteins in poplar (Populus trichocarpa L.) by using bioinformatic tools. Amino acid sequences of Dicer-like of the poplar and arabidopsis was received from the target databases by using BLAST tools. Then sequences were aligned by using the CLC Sequence Viewer 6 software. Continue domains, the phylogenetic tree and Three-dimensional structure of these sequences was plotted and finaly more features of these proteins determined. This study showed poplar has 7 Dicer-like protein Generally poplar Dicer-like proteins had PAZ, DEXDc, RIBOc and HELICc domains and according to the phylogenetic trees of Populus trichocarpa Dicer-like proteins are divided in four class. Weight and lengths of this proteiens respectively are 152 – 221 kDa and 1351-1967 amino acid. The results of the study could be a prelude for a more comprehensive study of these proteins in poplar.

Keywords: Dicer-Like proteins, Populus trichocarpa, Bioinformatics
Title:
Molecular dynamics simulation study of gramicidin like channel

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Abstract:
Design of peptides, using functional properties of natural ion channels, is a goal in development of novel macromolecular devices and therapeutics. A porin-like channel is a self-associated channel in membrane, composed of short β-sheet peptides. Gramicidin-like channel (GLC) is an ion channel synthesized recently by substitutions of D-alanine for glycines in the sequence of porin-like channel peptide. Existence of D-amino acid sequences makes it difficult to determine the exact secondary structure of GLC; however experimental methods such as CD and IR show a beta helical dimer for GLC similar to the structure of gramicidin A (GA) ion channel. GLC is an ion channel in membrane environment and is a head-to-head helical dimer. The GLC monomer was model built based on available GA dihedrals and used in MD simulations with CHARMM36 force field in GROMACS software to study its aqueous stability. Simulation in water in the presence of counter ions helped us to study the conformational motions of the peptide. In accordance with RMSD and radius of gyration analysis, the peptide was destabilized in water in agreement with the hydrophobic properties of side chains exposed to solvent. The RMSD and radius of gyration analysis are indicative of the level of compaction in the structure, i.e. how folded or unfolded the peptide is. Long time simulation studies confirm that the constructed peptide loses its proposed structure and become unfolded. As a future work, it is supposed to simulate GLC in POPC lipid bilayer. Therefore, we will evaluate peptide stability and lipid-peptide interactions in zwitterionic phospholipids.

Keywords: Antimicrobial Peptide, Molecular dynamics simulation, Beta helices, Gramicidin-like channel.
Title: Monolayers of bacteriorhodopsin on to FTO glass using Langmuir-blodgett (LB) technique

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Abstract:
Introduction: An organic thin film can be deposited on a solid substrate by various techniques such as self-assembly, thermal evaporation, electro deposition and Langmuir-Blodgett (LB) technique. The LB-technique is one of the most promising techniques for preparing such thin films as it enables the precise control of the monolayer thickness, homogeneous deposition of the monolayer over large areas and can be deposited on almost any kind of solid substrate. Molecular thin films were deposited by aligning bacteriorhodopsin (bR) on to FTO glass using the LB technique. Methods: The bR suspension was mixed with dimethylformamide (DMF) and water. The mixture was vigorously agitated and then this emulsion was carefully applied onto the aqueous surface (pH 5.5, at room temperature) to form a monolayer film of bR. After evaporation of the solvent, the monolayer of bR was compressed to the surface pressure of 20 mN/m, which was previously determined as the target pressure for optimal deposition of bR. The bR LB films were fabricated by depositing bR monolayer on to FTO glass with the dipping speed of 5 mm/min for the upward stroke. The absorption spectrum of bR LB films was measured to confirm the deposition of bR on to FTO glass. Results: Surface pressure–area per molecule (π–A) isotherms of bR monolayers at room temperature shows that stable monolayers of bR is formed at the air–water interface. The LB film deposition processes of bacteriorhodopsin are characterized by UV–vis spectroscopy. Conclusion: The results indicate that high quality and uniform bR film has been deposited on FTO glass. The bR monolayer films by LB technique can use for bioelectronics applications especially in bio-photocells and protein holographic memories.

Keywords: Langmuir-Blodgett, Bacteriorhodopsin, Protein monolayer
Title:
The investigation of Electron transfer mechanism of glucose oxidase at graphene and graphene oxide electrode

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Abstract:
Graphene, a two dimensional sheet of sp2 conjugated atomic carbon, has intense interest researching for unique properties such as high conductivity, ultrahigh carrier mobility and low cast. Graphene was used in many technologies such as sensors, solar batteries, super-capacitors, hydrogen storage and nanocomposite. Because graphene has a high conductivity, therefore it has many applications for electron transferring in redox protein study. In this work, graphen oxide was synthesis by chemical method, and then it was reduced to graphene. Graphene and graphene oxide was characterized by several spectroscopies, such as scanning microscope electron, infra red and UV-Vis spectroscopy. These data show that, graphene and graphene oxide was accessfully synthesis in nanometric scale. Also, the conductivity of graphene and graphene oxide was studied by electrochemical impedance spectroscopy. In next step, glucose oxidase was immobilized on graphene and graphene oxide by physical adsorption. The direct electron transfer of active site in glucose oxidase on graphene and graphene oxide was investigated by cyclic voltametry. Electrochemical impedance spectroscopy and cyclic voltametry show, the conductivity of graphene is high than graphene oxide. Thus, direct electron transferring of glucose oxidase on graphene is facile and its current is high, but glucose oxidase on graphene oxide not trasfer electron. Glucose oxidase reversibility on graphene is near to nernest potential. The formal potential of glucose oxidase is -240 mV in verses of Ag/ AgCl. Also, the thermodynamic and kinetic parameters such as electron transfer coeficient and electron transfer heterogeneity was extracted from different scan rate.

Keywords: glucose oxidase, graphene, electrochemistry
Title:
Spermine can modulate the abnormal changes of structure and function of fibrinogen at high glucose concentration

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Abstract:
Background: Fibrinogen is involved in hemostasis system and its abnormal function in type 2 diabetes is one of the important reasons for diabetic complications. Glycation of fibrinogen alters its structure and function and increases the risk of embolism in cardiovascular system of diabetic patients. L-Lysine as a chemical chaperone inhibits protein glycation and improves the diabetic complications. Therefore we investigated the effect of Lys on fibrinogen glycation.

Methods: Fibrinogen was incubated in the presence or absence of L-Lysine and high glucose concentration (50 to 400 mmol/L). The samples were retained for 4 months. The aliquots were given each two week. Then, they were analyzed by fluorescence spectroscopy, circular dichroism spectroscopy (CD), polyacryl amide gel electrophoresis (PAGE) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Its activity was also investigated.

Results: Structure and activity of fibrinogen were changed due to the glycation. L-Lysine as a chemical chaperone inhibited this phenomenon and the fluorescence emission, α helix and β sheet content, electrophoretic mobility and function of fibrinogen were closed to the normal values. Conclusions: Our results indicated a decrease in fibrinogen glycation by glucose in the presence of L-Lysine. It implies the usefulness of this amino acid in reduction of diabetes complications.

Keywords: Fibrinogen; High glucose concentration; L-Lysine; type 2 Diabetes; structure and function.
Title: Relatively Low Concentrations of Guanidine Thiocyanate Converts Lysozyme Native Structure to a Dominantly Beta Structure: A Circular Dichroic Analysis

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Abstract: Introduction: The deposition of naturally soluble proteins as amyloid fibrils underlies a large family of clinical disorders including Alzheimer's disease, the systemic amyloidosis and Type 2 diabetes disease. Chaotropic agents such as urea and guanidine hydrochloride are commonly used as protein denaturants in protein aggregation studies. Method: Here we investigated the effects of concentrated solutions of three different chemical denaturants, i.e., guanidine thiocyanate (GITC), guanidine hydrochloride (GdnHCl) and urea on aggregation properties of hen egg-white lysozyme (HEWL) by circular dichroism spectroscopy. Results: our results showed that incubation of lysozyme in different concentrations of denaturantes (0.5, 1, 2, 3, 4 and 5 M) resulted in rapid fibril formation and partial unfolding. The circular dichroic results showed that lysozyme molecules were converted to beta sheet structure at lower concentrations of guanidine thiocyanate (1 and 2 M) and at relatively high concentrations of guanidine hydrochloride (4 and 5 M). As we know conversion of native structure to dominantly beta structure is a determining stage in protein aggregation. Examination of different concentrations of urea showed that HEWL had lost some of its secondary structural elements in its native state, but still contained considerable α helical segments. Incubation of HEWL in different concentrations of urea did not result in any conversion to beta structure. Conclusion: Generally, we concluded that denaturation and destabilization that is exerted upon HEWL by GITC and GdnHCl is such that make them more appropriate in the investigation of HEWL aggregation and fibril formation.

Keywords: Lysozyme, Protein aggregation, Urea, Guanidine hydrochloride, Guanidine Thiocyanate
Title:
Inhibitors Of Acetylcholinesterase Reduce Aβ40 Aggregation Promoted By Butyrylcholinesterase: A Fluorescence And Atomic

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Abstract:
One of the hallmarks of Alzheimer Disease (AD) is the presence of senile plaques consisting primarily of amyloid β peptide (Aβ), a 40-42(3) amino acid containing polypeptide. Like acetylcholinesterase (AChE), butyrylcholinesterase (BChE) inactivates the neurotransmitter acetylcholine (ACh) and is hence could be of considerable importance as a potential therapeutic target in Alzheimer’s disease, which is a disease characterized by a cholinergic deficit and is treated by AChE inhibitors. In this study we compared the effects AChE inhibitors, fasciculine, propidium and galantamine on amyloid aggregation induced by amyloid-β peptide (Aβ1–40) in the presence and absence of BChE and/or AChE. Our results showed that BChE like AChE could promote Amyloid-β peptide aggregation. Also the presence of inhibitors, which interacted differently with BChE, in the reaction mixtures containing BChE reduced Aβ40 aggregation, initially promoted by BChE. Our results shows that BChE can promote Aβ1–40 aggregation (primarily non-fibrillar aggregates) which can be inhibited by AChE inhibitors although to different degrees. These kinds of studies is of potentially great importance in the design of new anti-cholinergic drugs with dual functions.

Keywords: Keywords: beta amyloid (Aβ), fibril formation, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), inhibitors.
Title:
Inhibition of Galectin-3 decreases invasion and adhesion but not migration of human ovarian cancer cell line SKOV-3

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Abstract:
Introduction: Galectin-3 (Gal-3) is known for its role in tumorigenesis and progression through regulating cell proliferation, apoptosis, cell adhesion, invasion, and metastasis by binding to the N-acetyllactosamine moiety of cell surface glycoproteins or glycolipids. SKOV-3 ovarian cancer cell line is highly metastatic and expresses Gal-3 but its function in ovarian cancer remains unknown. The aim of this study was to determine the role of Gal-3 in invasion and migration of SKOV-3 cells in the presence of Pectasol known as a competitive inhibitor of Gal-3 function.

Methods: Matrigel-coated transwells and wound healing assay were used for invasion and migration assay, respectively. Briefly, cells were treated for 24h with 0.1% and 0.5% pectasol, cells were stained with crystal violet and quantified. For adhesion test, pre-treated cells with pectasol for 16h were used, then trypsinized and after 30 min, 1 or 3 hours post seeding percent of adherent cells were quantified by using MTT test.

Results: Pectasol (0.1%) treated cells showed 2-fold higher number of migrated cells (P<.001) and 60% decreased number of invaded cells (P<.001) compared to control (untreated). However, cells treated with higher concentration of pectasol (0.5%) showed 50% decreased cell migration (P<0.05), while invasion showed 2-fold increase (P<.001) compared to control. Adhesion assay showed decreased percent of adherent cells in pre-treated cells with pectasol compared to control (p<0.05).

Conclusion: Our results suggest that Gal-3 is involved in migration and invasion of ovarian cancer cells in a dose dependent manner. Moreover, Gal-3 may increase ovarian cancer cell adhesion.

Keywords: ovarian cancer, Galectin-3, invasion, migration, adhesion
Title: 
The alteration of human serum albumin domains by sodium benzoate: Differential Scanning Calorimetry study

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Abstract: 
Introduction: Sodium benzoate is one of the preservative compounds which have many usages in different kind of industries like industrial food. This compound is oxidative and can change proteins structure and function. Regards to relationship between protein structural changes and thermodynamic parameters, sodium benzoate can cause thermodynamic parameter changes. The detection of thermodynamic parameters alteration is our aim in this research study.
Methods: We investigated sodium benzoate’s effect on Human serum albumin (HSA) after 35 days incubation using Nano Differential Scanning Calorimetry (N-DSC II). The results were analyzed by CpCalc software.
Result: Fresh HSA has one peak in its DSC profile and its Tm is equal to 64.1, but regards to our results, sodium benzoate caused a new peak formation beside the first peak in HSA profile which it’s Tm was 78.2 °C. Furthermore DSC results showed that HSA free energy (ΔG) and enthalpy (ΔH) were decreased by the effect of sodium benzoate (ΔG=3.6 , ΔH =183.3 ) compared with fresh- HSA (ΔG=6.5 , ΔH =205.3 ).
Conclusion: The reduction of free Gibbs energy implies that the stability of HSA was decreased; also the reduction in enthalpy indicates that sodium benzoate caused HSA loose its structure. Due to the results, partial unfolding was occurred in modified HSA after 35 days incubation period.

Keywords: Human serum albumin, sodium benzoate, differential scanning calorimetry, stability, structural changes
Title:
Interaction between Actinomycin D with G-quadruplex by Molecular Dynamic

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Abstract:
Introduction: G-quadruplexes are an attractive target for drug design because they found in biologically important regions. Formation of G-quadruplex in the purine-rich strand in the c-Myc promoter could block transcription of this oncogene. c-Myc is one of the best known oncogenes, and it is over expressed in various cancer cells. Several DNA binding ligands with antibiotic effects exhibited G-quadruplex binding activities. Here, interaction of c-Myc G-quadruplex with ActinomycinD (ActD) has been investigated by molecular dynamic simulation. ActD is a natural antibiotic and anticancer agent that inhibits the transcription of genes by interacting with a GC-rich duplex, a single-stranded or hairpin form of DNA, and then interfering with the action of RNA polymerase.

Method: In this study the complex formation between actinomycin-D and G-quadruplex is simulated by the Amber molecular dynamics package. The molecules were placed in a truncated octahedral TIP3P water box and neutralized by K+ ions. The salt concentration of 100 mM KCl was added to the simulation environment. We have performed Molecular Dynamic simulation to study interactions between ActD and the quadruplex.

Results: Analysis of molecular dynamics revealed that a very stable complex forms between the G-quadruplex and ActD, after a few nanoseconds. The major interactions responsible for stability are the hydrophobic interactions between the upper G-quartet surface and the ActD aromatic rings. The overall structure of the quadruplex is distorted in a way to engulf the lower part of the ActD molecule.

Conclusions: Anticancer effect of ActD can be related to its binding to c-Myc G-quadruplex structure.

Keywords: Molecular Dynamic, Actinomycin D, G-quadruplex, Interaction
Title: study of lysozyme amyloid fibril formation in the presence of taxol as a polyphenol inhibitor

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Abstract: Introduction: Alzheimer disease and some other neurodegenerative diseases are occurred because of a kind of protein aggregation called amyloid fibrils. Since formation of amyloid fibrils is cytotoxic, effort to inhibit this phenomenon is considerable. Some studies have shown that small molecules, polyphenols and peptides can act as fibril formation inhibitors. In the present study, taxol is used as a polyphenolic small molecule, in order to inhibit amyloid fibril formation of lysozyme.

Methods: Acidic pH is used to form amyloid fibrils of lysozyme. ThT fluorescence, Congo red absorbance, circular dichroism and atomic force microscope (AFM) are used to study formation of lysozyme fibrils without and with taxol in ratios of 10:1, 100:1 and 1000:1 (P:L).

Results: In the present of taxol, ThT fluorescence and Congo red absorbance are reduced. this reduction is in direction with increase of taxol concentration. Secondary structure of lysozyme is remained near to native in higher concentration of taxol. AFM study shows morphology of fibrils is disrupted in the presence of taxol.

Conclusions: In acidic pH, lysozyme structure become partial unfolded and hydrophobic core expose, that trigger aggregation of lysozyme. taxol interaction with partial unfold lysozyme, prevents interaction of hydrophobic parts of lysozyme and aggregation.

Keywords: lysozyme, amyloid fibril, taxol, inhibition.
Title: Decreasing hemoglobin glycation in a diabetic condition by bee venom

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Abstract: Proteins are exposed to a lot of altering factors during their lifetime. Sugars are one of these factors that cause glycation in proteins. Protein glycation is initiated by a nucleophilic addition reaction between a free amino group from a protein and a carbonyl group from a reducing sugar. This reaction leads to structural alterations at the secondary and tertiary levels and consequently functional properties of the protein. Bee venom has some therapeutic properties and traditionally used in treatment of various diseases. The hypothesis of this study was, if bee venom decreases the amount of glycation in hemoglobin, it can be used as a treatment of glycation in a diabetic condition. Hemoglobin was incubated in the presence and absence of glucose and bee venom for 5 weeks. The number of free lysine residues in the protein was determined using the fluorescamine, which reacts with free amino groups, and in this state has fluorescence characteristic. UV–vis absorption spectra of soret band were measured by a Shimadzu ultraviolet spectrophotometer. Fluorescamine emission results showed that the percentage of free amino group of lysine in glycated hemoglobin decreased significantly in the presence of glucose. Also bee venom increased significantly the amount of free amino groups. Glycation leads to decrease in the soret band absorption, whereas in the presence of bee venom the absorption of soret band was increased. Some effective antiglycation agents have been synthesized but investigations stopped in the clinical trials because of their side effects. Our study showed that bee venom is a natural antiglycation agent and it can be developed as a new drug in diabetes treatment.

Keywords: Diabetes, Glycation, Hemoglobin, Bee venom
Title:
Spectroscopic methods to accurate and fast study of kinetic parameters of DNA cleavage DNAzyme

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Abstract:
The Cu$^{2+}$ dependent DNA cleavage DNAzyme isthe unique example of known DNAzymes. The most established method for measuring the kinetics of deoxyribozyme reactions is based on the use of radiolabeled substrates. This method entails the cumbersome sampling, electrophoretic separation, and quantitation of the reaction products, as well as the continuous loss of substrate due to decay of the radioactive label. In this study werepresent accurate, fast and inexpensive methods for kinetic study of the DNAzyme which is based on spectroscopic techniques. One of these methods is based on the SYBR gold extrinsic fluorescence. This probe has high affinity to double stranded DNA compared to single stranded DNA. Continuous Hyperchromicity assay (CHA) is used in second method which is a function of DNA hyperchromicity that observed when double stranded DNA transformed to single stranded DNA. Using this methods can be traced DNAzyme substrate hybridization and product formation simultaneously with reaction progress. Under conditions where the product release faster than enzymatic reaction, production release speed which is traced with these methods determined enzymatic reaction kinetic. These methods in addition to the kinetic study of the DNA cleavage DNAzyme can be used to study of other DNAzymes that has nucleic acid substrate. Comparison of the results show that the continuous hyperchromicity assay is more accurate than the extrinsic fluorescence method using SYBR gold because CHA method is DNA intrinsic physicochemical property result without interference of disturbing factors such as SYBR gold.

Keywords: DNAzyme, continuous hyperchromicity assay, extrinsic fluorescence, kinetic
Title:
Effect of selenium on the blood serum's acetylcholinesterase activity under Extremely Low Frequency Electromagnetic Fields (ELF-EMF)

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Abstract:
Introduction: Exposures to many ELF-EMF are increasing significantly as technology advances unabated and new applications are found. The effects of electromagnetic fields on living microorganisms have been investigated for a long time. Various studies showed that ELF-EMF affect some biochemical processes and result in changing some serum biochemical parameters and enzymes. In this study, the short time effects of ELF-EMF with the frequency of 100 Hz, 0.3mT on the blood serum’s acetylcholinesterase activity is investigated in the female mice. For this purpose, four groups of mice were divided into the control group (group I; animals with normal feeding diet without selenium complimentary) and the experimental group (group II; animals with normal feeding diet with selenium complimentary) (n=5 each). Then all animals were exposed to a power off EMF for four hours a day, in the period of five days. Acetylcholinesterase activity was measured with UV-vis spectroscopy. Results: The results indicate that the blood serum’s activity of acetylcholinesterase under ELF-EMF was significantly higher in the control group than in experimental group. Conclusions: In conclusion, the selenium has non-protective effects on the blood serum’s acetylcholinesterase activity under ELF-EMF.

Keywords: Selenium, Extremely Low Frequency Electromagnetic Fields, Acetylcholinesterase, Mice
Title:
Structural changes and enzymatic activity of RNase A in the presence of cationic surfactants

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Abstract:
Bovine pancreatic ribonuclease A (RNase A) is an endoribonuclease that cleaves and hydrolysis the single stranded RNA in two distinct steps. This enzyme has played a crucial role as a model system in studies of protein structure, folding and unfolding pathways and enzyme catalysis. So far extensive studies have been carried out on the structure and function of this enzyme. The purpose of this study was to characterize the changes of structure and enzyme kinetics of RNase A by cationic surfactants. The structural changes of RNase A have been investigated initially in the presence of a homologous series of cationic surfactants namely (dodecyltrimethylammonium bromide (DTAB), tetradecyltrimethylammonium bromide (TTAB), and hexadecyltrimethylammonium bromide (HTAB)) at pH=5, using UV-Vis and fluorescence techniques. Titration curves of RNase A in the presence of different concentrations of surfactants were also analyzed. Results of this section indicated that cationic surfactants cannot denature RNase A. The second phase of this study, kinetics reaction of RNase A with Cytidine 2'-3'-cyclic monophosphate as substrate, was investigated by UV-Vis spectroscopy and Michaelis-Menten parameters were extracted. Comparison of kinetics parameters ("V" _"max" , "K" _"m" and "K" _"cat" ) showed, influence of cationic surfactants DTAB, TTAB and HTAB on kinetics enzymatic reaction was restricted.

Keywords: Cationic surfactant, Ribonuclease A, Enzyme Kinetics, Kinetics parameters, Activation, Inhibition
Title:
Determination IC50 of many natural small compound on porcine pancreatic alpha amylase

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Abstract:
Introduction: Alpha-amylase catalyzes the hydrolysis of α-(1,4)-D-glycosidic linkages of starch and other glucose polymers. Inhibitors of this enzyme could be use in the treatment of obesity and diabetic patients. Inhibitors of alpha-amylase include proteinaceous and carbohydrate-like structures. In recent years, small molecules have also been studied in this regard. In this studied IC50 of many small molecules toward porcine pancreatic alpha amylase (PPA) were determined. Method: One of the methods that we use for enzyme assay is bernfeld method. In this way, the best time for incubated is 30 minutes and we should previously did it in the presence or absence of inhibitor. Most of the inhibitors were dissolved in DMSO before use, and the control sample contained the same amount of DMSO. Results: IC50s of these compounds(µM) toward PPA were found to be as follows: Eugenol=1138.5, Vanillylacetone=1898.8, diethylstilbesterol=1860.2, Estragol=1772.2, Trans-anethol=202.8, Citral=120.8, Trans-chalcone=96.44, Thioflavin-T=93.75. Conclusion: Therefore thiflavin-T, trans-chalcone and citral with IC50 about 100µM are as strong inhibitor, trans-anethol with IC50 about 200µM is a moderate inhibitor and vanillylacetone, diethylstilbesterol, Estragol, eugenol with IC50 between 2000-2000µM are weak inhibitor.

Keywords: KEYWORDS: Alpha-amylase, Inhibitor, Starch, Enzyme.
Title:
Stem anatomical study of three Erodium (Geraniaceae) species in Iran

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Abstract:
ABSTRACT:
Introduction: Geraniaceae is composed of 5 genera and 750 species which are somehow cosmopolitan. Erodium is a genus with 62 species in Mediterranean region. In Iran there are 14 species of this genus.
Aim: in this study stem anatomical study was done for three species as Erodium ciconium, E. gruinum, E. malacoides for the first time.
Methods and material: for stem anatomical study 3 accessions were sampled for each species for their natural habitats of Iran. Middle part of stem from third internode was studied by hand-made cross section and double coloration.
Result: based on quantitative and qualitative as length and width of stem cross section, dimensions of vascular bundles, fibre thickness, general shape of stem section, collenshymas thickness, and shape of outer layer of stem sections, hair types, number and shape of vascular bundles species relations were evaluated.
Conclusion: three studied species are clearly separated based on selected features.

Keywords: Geraniaceae, Erodium, Stem anatomy
Title:
characterization and Cytotoxicity of Platinum Anticancer Complex Encapsulated in Beta-Casein-Chitosan Nanoparticles

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Abstract:
Introduction: Clinical application of platinum-based anticancer drugs is largely limited by severe toxicity. Drug delivery systems are highly desired for improving the efficacy and applicability of these drugs. This study describes an alternative strategy for the delivery of a new synthesized Pt(II) complex (bipyridin morpholin dithio carbomate Pt(II) nitrate) by its encapsulating in the Beta-Casein (B-CN)-Chitosan(CS) nanoparticles. Beta-casein, an amphiphilic milk protein, self-associates into micelles in aqueous solutions.

Methods: Initially, the influence of pH on the formation of colloidally stable nanocarrier system composed of Pt complex-loaded B-CN and chitosan nanoparticles was investigated using dynamic light scattering (DLS) and scanning electron microscopy (SEM). Then, the cytotoxicity of free and encapsulated Pt complex were evaluated on colorectal carcinoma HCT116 cells.

Results: Formed Pt complex-loaded beta-casein-chitosan nanoparticles were stable and soluble at the pHs of 5.7 and 6.2. DLS data showed that the particles formed had sizes between 200 and 300 nm. At these pHs, the values of zeta-potential of nanoparticles were positive and formed particles were stable. SEM analysis confirmed the formation of nanoparticles with a good colloidal stability and an average particle size of 200 nm. Both cytotoxicity and cellular uptake of platinum were enhanced by its entrapping in B-CN-CS nanovehicles.

Conclusions: These findings suggested that this novel drug-delivery system allows drugs to be thermodynamically stable in aqueous solutions and potentially useful for targeted drug-delivery applications.

Keywords: Beta-casein, Platinum complex, Chitosan, nanovehicles, drug delivery system
Title:
Live-imaging of Zebrafish mid-blastula transition (MBT) stage development: Effect of temperature

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Abstract:
Introduction: It is well known that a reduction in water temperature can delay Zebrafish embryonic development. In case of Zebrafish, lowering the temperature from 27°C to 16°C can withstand mid-blastula (MBT) developmental arrest up to 4 hours. However, it is not clear how fast the development can be proceed after returning the embryo from 16°C to 27°C. Therefore, in these studies, we have calculated the speed of developmental recovery. Method: Zebrafish maintenance and breeding were performed following standard procedure. Fertilized embryos were collected before they attend MBT stages. Embryos were cleaned and embryos of 4 hour post-fertilization (hpf) were collected following morphological diagramed provided by Kimmel et al 19952. The embryos were then either transfer to 27°C (control) or 16°C (treated) for three hours and another one and haft hour in 27°C for recovery. The whole procedure was carried under microscope fitted with CCD camera and the live-image of development were capture by image capturing software. Results: Development of Zebrafish take place in plan progressive manners at normal temperature (27°C). However, in mid-blastula stages, the development could be arrested for certain period of time (our case 3 hours) at lower temperature of 16°C and below that could be recovered by re-incubating the arrested embryos at 27°C. However, the speed of recovery is greatly dependent on the arresting temperature. The rate of recovery is similar to normal speed (but not the stages of development) for the embryos arrested at 16°C. Lowering the temperature below causes drastic reduction in recovery speed. The embryos arrested at 4°C, shows less than 25% of recovery speed compared to normal. SDS-PAGE analysis of soluble fraction of embryo lysate shows that the embryos arrested at 4°C shows drastic reduction of a prominent band (above 97 kD) that were present before arrest while embryos arrested at 16°C, this band remain unchanged. Further SDS-PAGE analysis of soluble fraction of embryos lysate show a gradual decrease in the same protein band indicating this protein band is an indicator of developmental stage and is temperature sensitive.

Keywords: Zebrafish, Live-imaging, Embryonic development.
Title:
Comparison of C3 and C4 with CH50 in zanjan medical diagnostic laboratory Patients

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Abstract:
Introduction:
The complement system comprises a group of serum proteins and cell membrane receptors
that function primarily to fight infection. These components interact in three activation pathways.
The central results of activation of these pathways are to deposit the opsonin C3b on bacteria to
promote phagocytosis, to lyse bacteria by the assembly of the terminal membrane attack
complex and to promote inflammation. The aim of this study was to compare the rate of
increase or decrease in C3 and C4 in patients is CH50.

Methods:
This Cross-sectional study on 121 patients referred to the Zanjan's Clinical Laboratory, C3 and
C4 were measured by nephelometry method, CH50 levels were measured using SRID. Then
the data were analyzed by the SPSS19 Software.

Results:
The results of tests on patients revealed that, 6.72 percent with high C3, the 21.01 percent with
low C3, 7.56 percent with high C4 and 12.61 percent of these patients had low C4. the CH50
data not showed a significant increase or decrease in visitors.

Conclusions:
This study showed that CH50 measurements alone can not be the expression of the
complement system function. And other measures such as the complement component like
C1q, C3, C4, C5, and C9 with CH50 recommended.

Keywords: C3, C4, CH50, Zanjan
Title:
Effect of temperature on the activity of Bacteriorhodopsin as a candidate for nano protein memory

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Abstract:
Introduction: Biological and physical Laws have created the newly scientific movement in the electronics and its allied industries by discovering a newly developed storage solution. Technique involving in the information storage at the molecular level has received special attention in order to increase the capacity and to speed up the data storage. In this technique each molecule plays a role as storage device. Optical storage of information at the molecular level can be performed by using photo reversible materials. Using optical storage is effectively applicable for data processing, because of high rate of chemical reactions of photosynthesis. Bacteriorhodopsin(bR) is a protein with simple optical system in which the variety in chemical states is observed. In present study the effect of protein activity on both bR suspension and bR in a film pre-coated on Polycarbonate Groove was evaluated at different temperatures.

Method: At the first step of experiment, Gelatin-polyvinyl alcohol matrixes weight/volume 1% as a film and bR 3.2 mg/ml were prepared. Then the protein activity in different temperature conditions was examined by measuring changes in the pH.

Result: The best action in bR suspension was observed at temperatures below 20 °C. However, the bR in film pre-coated on Polycarbonate Groove obtained the best action at temperatures above 60 °C.

Conclusion: These two samples have received appropriate activities at different temperatures ranging between 4 and 65 °C. The results revealed that bR suspension had the best activity at temperatures below 10 °C, on the other hand, bR in film pre-coated on Polycarbonate Groove had the best activity at temperatures above 65 °C.

Keywords: Bacteriorhodopsin, temperature, nano protein memory
Title:
Fasting inhibits advanced glycation end products (AGEs) in human serum albumin upon incubation with 3-β-hydroxybutyrate

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Abstract:
Introduction: The ketone bodies are metabolites which are produced through metabolism of fatty acids in the liver when glucose is not readily available. The 3-β-hydroxybutyrate is one of the main types of ketone bodies that are about 78% of all ketone bodies. The metabolism of fatty acids increases during fasting, prolonged exercises and in diabetic (type I) patients, so enhances the concentration of 3-β-hydroxybutyrate in the body. In this study, the inhibitory effect of 3-β-hydroxybutyrate on advanced glycation end products (AGEs) formation by glucose from the human serum albumin (HSA) was studied at physiological conditions after 35 days of incubation.

Methods: In this research, used two techniques circular dichroism (CD), fluorescence spectroscopy and differential scanning calorimetry (DSC).

Results: The result of CD indicated that the secondary structure of modified HSA (by combination of glucose and 3-β-hydroxybutyrate) in spite of glycated HSA was not changed. In addition, AGEs fluorescence intensity measurements showed that AGEs product formation with 3-β-hydroxybutyrate was decreased. These results analyzed by DSC.

Conclusion: In sum up, 3-β-hydroxybutyrate can restrain the structural changes of glycated HSA and inhibit AGEs formation. Fasting and prolonged exercises increase 3-β-hydroxybutyrate in the blood, so it is a good inhibitor for some pathological disorders and physiological aging.

Keywords: Human serum albumin, 3-β-hydroxybutyrate, ketone body, differential scanning calorimetry, advanced glycation end products inhibitor.
Title:
Cloning and expression of the variable regions of anti-EGFR monoclonal antibody in E.coli for production of single chain antibody

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Abstract:
Introduction: EGFR overexpression is characteristic of various malignancies and could be considered as an excellent object for designing specific inhibitors such as anti-EGFR monoclonal antibodies (mAb) for cancer therapy. Drawbacks exerted by large size of fulllength antibodies leads to development of single chain antibodies that benefits from smaller size and short circulation half-lives. In this paper, we describe the engineering of monoclonal antibody C225 into a single chain form and evaluation of its reactivity with cells expressing high level of EGFR.

Method: The RNA extracted from C225 cells was reverse transcribed to cDNA and used for PCR amplification of genes encoding light and heavy chains variable regions. The PCR products were cloned and expressed in E. coli BL21 for production. The expressed protein was analyzed by SDS-PAGE and purified by Ni-NTA affinity chromatography. Analysis of reactivity of purified C225-scFv with EGFR expressing A431 tumor cell line was tested by westernblotting and enzyme-linked immunosorbent assay.

Results: The results indicated that C225-scFvs are highly expressed in E. coli appeared as a 26 kDa protein in SDS-PAGE analysis of induced cells. Analysis of reactivity of purified C225-scFv with A431 tumor cell line by westernblotting and ELISA revealed high affinity binding of recombinant C225-scFv to target cells.

Conclusions: In conclusion the results of this study indicated that C225-scFv produced in this study is capable to bind to EGFR and according to previous studies it could be used in diagnosis and treatment of EGFR over-expressing tumoral cells.

Keywords: Single Chain Antibody, EGFR, MAb C225
Title:
Effect of different carbon sources on the activity of catalase in pseudomonas aeruginosa

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Abstract:
Catalase is an important enzyme that is responsible to break down H$_2$O$_2$ in the cells. *Pseudomonas aeruginosa* is a Gram-negative and rod shape bacterium known as a hydrocarbon-using microorganism. In the present work the kinetics parameters of the enzyme in media containing crude oil, hexadecan, an ethanol were compared with control medium containing glucose. *Pseudomonas* was cultured in liquid media containing the above carbon sources. As the early of stationary phase the cells were harvested and cell free extract was prepared. Our result showed that the $V_{\text{max}}$ of enzyme were highest in glucose ($V_{\text{max}}$ = 17.24 µmol/min/mg protein and $K_m$ = 0.45mM) while it was lowest in hexadecan ($V_{\text{max}}$ = 9.09 µmol/min/mg protein and $K_m$ = 0.6mM). The $K_m$ and $V_{\text{max}}$ of enzyme in ethanol and crude oil were 1 mM, 16.6 µmol/min/mg protein and 0.28 mM, 11.90 µmol/min/mg protein respectively. The spesific activity of enzyme was also highest in ethanol 9.65 U/mg protein while it was lowest in glucose 2.01 U/mg protein. The spesific activity of enzyme in hexadecan and crude oil was 7.5 U/mg protein and 3.58 U/mg protein. our results suggest that in different carbon sources the bacterium can affect the enzyme activity and kinetic parameters.

Keywords: catalase - pseudomonas - kinetics parameters - enzyme
Title: Investigation of total lipid production by Mucor hiemalis PTCC 5292 in different media cultures.

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Abstract: Introduction

Lindner’s group in Germany during World War I were conducted first trials on the commercial production of microbial oil. Microorganisms that, accumulate lipids more than 20% of their biomass are called oleaginous microorganisms and among them fungi are the best group of valuable fatty acids producers. Microbial lipids has been considered due to the microorganisms cheap and easy growth conditions, high growth rate, safety and production of valuable and different lipids with good physiological functions in human body. In this study, the efficiency of total lipid production was investigated by Mucor hiemalis PTCC 5292 in different media cultures.

Method

M.hiemalis was grown on PDA at 25°C for 7 days. 1 X 10^7 spores were inoculated on 50 ml of media A (glucose, yeast extract) or B (glucose, yeast extract and different mineral salts) in 250 ml Erlenmeyer flask. The media cultures were grown at 25°C with shaking at 150 rpm for 72h. Total lipid, dry biomass, glucose and pH measured at 24, 48, 72 and 96h. Lipid extraction of mycelia were according to the modified procedure of Bligh and Dyer (2009).

Result

The total lipid content of media A and B were 41% and 58% respectively. Maximum production occured in 72h. Glucose and pH curves along the time confirmed our results.

Conclusion

The results showed that, media B was more suitable for lipid production comparing to media A and high yield of lipid production in 72h. By extended research the total lipid production in M.hiemalis is optimized. In addition, the fatty acid profile and it’s quantity is investigated.

Keywords: Mucor hiemalis, media culture, total lipid, lipid extraction
Title: A Computational Study on Anticancer Drug Camptothecin Interaction with Single Walled Carbon Nanotube

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Abstract: Camptothecin demonstrated strong anticancer activity in preliminary clinical trials but also low solubility and adverse drug reaction. CPT is believed to be a potent topoisomerase inhibitor that interferes with the essential function of topoisomerase in DNA replication. Since carbon nanotubes discovery in 1991 by Iijima, it have been considered as the ideal material for a variety of applications. Carbon nanotubes own unique sets of properties such as biocompatibility in pharmaceutical drug delivery systems, they also play an excellent role as drug carries with a highly site-selective delivery and sensitivity for particular diseases like cancer. This work reports an investigation of the combination of CPT and SWCNTs with DFTB method. We calculated stabilization energies and physiochemical properties. The results show the interaction of CPT and SWCNTs has stabilization energies and structural stability in solvent. What we think is that our results are helpful for drug design and drug delivery.

Keywords: Camptothecin, SWCNTs, DFTB, AntiCancer drug
Title:
Effects of Different metals on activity of wild type and mutant pyrazinamidases

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Abstract:
Introduction:
*M. tuberculosis* pyrazinamidase (encoded by the pncA gene) is a metalloprotein that in its metal binding site was found to hold a Fe$^{2+}$ ion coordinated by one aspartate (Asp49) and three histidines (His51, His57, His71). Pyrazinamidase (PncA) activates the first-line antituberculous drug pyrazinamide into pyrazinoic acid. Emergence of strains resistant to PZA linked to mutations in the pncA gene. The concomitant mutations can decrease hydrolytic activity by structure changing that can affect on metal binding site. In order to first we detected how mutations effect on mutant pyrazinamidases activity in absence and > metal ions.

Methods:Wild type and mutant PZAses were cloned and expressed. Their activities were assayed by a qualitative colorimetric method according to Wayne test and presence of a red color detected by visual inspection. Then enzyme activities were determined in Mg$^{2+}$, Ni$^{2+}$, Fe$^{2+}$ ions, at a final concentration of 1 and 2 mM. Our assay results indicated that 1 of 3 recombinant PZAses of PZA-resistant M. tuberculosis clinical isolates (containing T160P) had almost no activity, whereas other PZAses of PZA resistant isolates displayed less activity. Also the results obtained from this study indicated enzymatic activity of recombinant PZAses can be increased by Ni$^{2+}$ while in the presence of other metals activity of different recombinant PZAses indicated no or less changing.

Conclusion:These results suggest that mutations in recombinant PZAses altered the enzymatic activity according to the localization of the mutation and the type of substitution and also some metals can increase mutant pyrazinamidases activity by more effective interactions.

Keywords: M. tuberculosis, Pyrazinamidase, Pyrazynamide, Mutation, Resistance
Title:
Biomedical Optical Imaging for early diagnosis using Laser Sources

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Abstract:
During the last two decades a wide range of new methods and techniques of photonics in biomedical imaging in order to early diagnosis of diseases have been developed. These methods use extracted quantitative information and data from optical images to find in vivo physiological status of the tissue. Using optical methods and technologies for biomedical applications in diagnostics and therapeutics has come into view as a major research field. Currently, the optical diagnostic in medicine, considered a part of field of medical diagnostic techniques, has three branches undergoing of development worldwide: a) Optical molecular imaging techniques b) The highly developed real-time for Two- and Three-dimensional imaging and Optical Coherence Tomography OCT techniques, c) Nano- and microscale techniques in medical laboratory diagnostics and analytics. These fields are completed using image processing techniques as part of medical diagnostic imaging. In this presentation, the common basis of the techniques which use optical spectrum to image live tissue and the development of the technologies and methods in the last decade will be explain. What the current international works are and how are the movements in this field. We introduce our new non-invasive real-time imaging technique using Aberdeen scanning laser which is developing and adapting for early detection of ischemic diseases such as diabetic retinopathy (DR). We recommend current techniques may be replaced in some eye imaging fields by this new method, to help DR patients so decrease treatment costs and enhance public health.

Keywords: Biomedical Optical imaging, laser sources, tissue imaging, Spectral imaging, early diagnosis
Study of heterozygosity of rs1799989 in TYR gene

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Introduction: Tyrosinase is the most important enzyme in the production of pigments of the skin, eyes and hair follicles. The enzyme is encoded by tyrosinase (TYR) or Oculocutaneous Albinism (OCA1A) gene. Mutations in TYR gene result in pigmentary disorders such as Albinism in human. Investigation for informative markers, located in the TYR gene region is an important step in linkage analysis and molecular diagnosis of the disease in Iranian population.

Method: In present study by use of different bioinformatic tools, a single nucleotide polymorphic marker (rs1799989) at the 5' region of the TYR gene were investigated. After that, four primers for the purpose of genotyping was designed. DNA was extracted from the peripheral blood of 110 unrelated individuals and 30 family members of control Iranian people and amplified by Tetra ARMS PCR technique.

Result: The result of DNA amplification were analyzed on Agarose gel. After genotyping of the marker, allele frequency and heterozygosity of the marker were obtained.

Conclusion: Given the importance of the 5' region of TYR gene and the presence of different mutations involved in the Albinism phenotype, introduction of informative molecular markers in this region and genotyping them, could be useful to determine their phase and heterozygosity and following, molecular diagnosis of the disease in the Iranian population.

Keywords: Tyrosinase gene, rs1799989, Tetra ARMS PCR, OCA1A
Title: Effects of lorazepam and midazolam on the structure of adenosine deaminase: Molecular dynamic studies

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Abstract: Effects of midazolam and lorazepam on the structure of adenosine deaminase (ADA) were studied by molecular dynamics simulation. Adenosine deaminase (ADA) is a cytosolic enzyme, which has been the object of considerable interest, mainly because a congenital defect in the enzyme causes severe combined immunodeficiency disease (SCID) in humans. All calculations were done with gromacs software and Gromose 96 43a1 force field at 300 K. The studied number of midazolam and lorazepam were 6 and 12 molecules. Accessible surface area (ASA), radial distribution function (RDF) and other physical parameters were obtained from analyzing trajectory of molecular dynamics. Results demonstrated that by increasing drug concentration, protein denaturation increase too, therefore enzyme denaturation is more in the presence of higher concentration of drugs. Increase of solvent accessible surface area and protein radius of gyration are good indicators of protein denaturation by these molecules. Variations of helix, beta and coil were analyzed using topology and trajectory files by the VADAR software. Analyzing the secondary structure revealed that both molecules decrease the secondary structure and lorazepam is more effective due to having a smaller size. Therefore increase of beta and coil with decrease of helix structure was observed which confirms simulation results.

Keywords: Molecular dynamic simulation, Midazolam, Lorazepam, Denaturation, ADA
Title: Differential regulated expression of DRP in human Astrocytoma tumor discovered by a proteomics analysis

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Abstract: Scientific data about protein biomarkers which can be used in distinguishing tumor from non tumor brain tissues are increasing. These proteins might be attractive clinical biomarkers, linked or not with survival, or a specific cancer-therapy target. Dihydropyrimidinase proteins (DRP) are members of collapsing response mediator proteins (CRMP); involved in regulation of neuritis guidance and synapse formation. CAMP family members, (DRP 1-5) have been essentially studied in the context of neurodegenerative diseases. They are highly expressed during brain development and rarely in adult brains. In an attempt to get an understanding about functional proteins of Astrocytoma tumor, proteins extracted from tumor and normal brain tissues and then evaluated the protein purity by Bradford test and Spectrophotometery method. In this study, proteins separated by 2D-DIGE method and the spots were then analyzed and compared using statistical data and specific software, after providing 3D images of spots alteration. Spots were identified by PI, molecular weights and data banks. Results revealing, DRP2 and DRP3 were identified as differentially expressed in Astrocytomas. Post translational modification such as myristilation, methylation or esterification can alter the PI of the proteins and glyconylation and prenylation can alter their molecular weight the DRP family is known to be highly phosphorylation.

Keywords: DRP, Astrocytoma, 2D-DIGE and Brain Tumor
Title:
Differential protein expression Cluster and Principal Component Analysis in Astrocytoma proteome

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Abstract:
Astrocytomas are human brain tumors that arise form Astrocytes, the most abundant type of Glial cells of the Central Nervous System, or more likely, form resident cancer stem cells. Proteomics analysis in now applied widely in every area of neuroscience research including brain cancer. The paper critically evaluates the potential of two distinct strategies, functional Clustering an Principal Component Analysis, to better interpret Astrocytoma proteomics data. We extracted proteins of tumor and normal brain tissues and then evaluated the protein purity by Bradford test and Spectrophotometery method. In this study, we separated proteins by 2DG Electrophoresis method and spots were then analyzed and compared using statistical data. Results demonstrate that functional Clustering and Principal Component Analysis each have considerable merits in aiding interpretation of proteomic data. Cluster and Principal Component Analysis that may be co-regulated and play a role in human Astrocytoma tumor. Two approches highlight (1) subgroups of proteins that may be co-regulated and play a role in Astrocytoma tumor, and (2) functional protein interactions that may improve comprehend sion of biological mechanisms involved.

Keywords: Proteomics, Cluster, Astrocytoma and 2D-DIGE
Title:
Charaterization of crude xylanase produced by edible mushroom Pleurotus eringii

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Abstract:
Xylanases has been increasingly forthcoming in recent years because of their possible involvement in numerous industrial processes including bioconversion of lignocellulose drove sugars in to fuels, processing food and the paper and fibre industries. Edible mushrooms are emerging as important source of xylanolytic enzymes and this study has concentrated to produce and characterize xylanases by Pleurotus eringii. The crude enzyme was characterized on the basis of various parameters such as incubation time, substrate specificity, substrate concentration, enzyme volume, buffer, pH, pH stability, temperature, temperature stability, and effect of various metal ions or compounds. The xylanase activity was noted maximum at 15 minutes of incubation time, 2.0% xylan and 0.5 ml enzyme volume. The highest enzyme activity was found at pH 4.5, whereas xylanase exhibited maximum stability in the range of pH 4.0 to 10.0. The maximum xylanase activity was noted at 60°C, while enzyme was most active and retains more than 40% activity at 90°C with in 10 minutes of incubation. ZnCl2 (10mM) stimulated the xylanase activity as compare to other metal ions or compounds. It is concluded that Pleurotus eringii is capable to produce pH stable and thermostable xylanase for industrial purposes.

Keywords: characterization, xylanase, edible mushroom Pleurotus eringii
Title:
Complexation of of Iron(II) and Copper(II) with Levodopa and its analogs; Main Contents of Antiparkinsonian Drug

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Abstract:
Levodopa, Carbidopa and Methyldopa are dopamine analogs present in antiparkinsonian (neurodisorder) drugs. Dopamine contains catecholic functional group due to which it is liable to interact with substances present in the physiological system. Iron which has significant role in the biological system and is required for normal brain and nerve function through its involvement in cellular metabolism is assumed to bind through catecholic site of the molecules and lower the efficiency of administrated drug or this interaction of iron to dopamine could either result in over accumulation of iron which may lead to several hazardous effects. The Chelation of Bio-Essential Metal Iron and Copper with Levodopa and it’s analogs was studied at 30±2°C. Fe^{2+} formed intense color complex with these molecules with an absorbance maxima in visible region. A distinct peak of Fe-Carbidopa complex at 364 nm was observed while that of Fe-Levodopa and Fe-methyldopa are observed at 730 nm. Where as Cu^{2+} showed absorbance maxima at 525 nm, Cu^{2+}-LD and Cu^{2+}-MD did not show any significant absorbance in visible region so it’s interaction is studied using potentiometric technique. Potentiometric studies provided evidence for chelation of dopamine molecules with Fe^{2+} and Cu^{2+}. Stoichiometry of Fe(II) with these dopamine analogs has been explored which shows that Fe(II) forms a 1:3 complex with levodopa and methyl dopa while a 1:2 complex is found in case of carbidopa. An ML_2 complex is formed in case of Cu^{2+}-CD. References 1. Peciña S, Berridge K (2005). J Neurosci 25(50). 2. Lemke M, Brecht H, Koester J, Kraus P, Reichmann H (2005). J Neuropsychiatry Clin Neurosci 17 (2): 214–20. 3. Fatima N, Zaidi S. Z. A, Nisar S, Qadri M (2013), Pak. J. Chem., 3(1), 1-6.

Keywords: Levodopa, Iron(II), Copper(II), potentiometry, stoichiometry,
investigating alpha-beta transition in prion proteins using beta_lacto globulin as a model

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Abstract:
introduction:Beta-lacto globulin(blg) is a milk proteins which has 162 residues and has many beta-sheets.Using alchohol can denature blg.Unfolded state of blg is almost helical.By removing alchohol we can induce the refolding of this protein.During refolding process, an alph-beta transition occurs thus we can use blg as a model to understand the mechanism of alpha-beta transition in proteins such as prions.Prions(prp) are ordinary proteins of nervous system.prps are alpha-helical almost, however because of some reasons they change into beta-sheets and starts to aggregate in nervous cells.This aggregation is neurodegenerative and results in prion diseases.Amino acid sequence of prp is available however its 3D structure has not yet been achieved.Much researches on NMR structure of prp currently is done to find the mechanism of alpha-beta transition of these proteins but we think that this transition is most related to environmental factors rather their structure.Thus we use blg in our computational analysis to understand this mechanism because blg 3D structure is available and also shows the alpha-beta transition.

methods:We used blastp to find similarity between blg and prp.Genebank at national center of biotechnology helped us to find mutations then we could find a meaning linkage between blg and mutations of these proteins.

results:Using blastp showed no similarity between blg and prp,thus this transition cannot be related with protein structure and is in accordance with environmental factors such as PH.

conclusions:The undetermined 3D structure of prion is a great obstacle and by characterizing this structure we can understand the precise mechanism of alpha-beta transition.

Keywords: blg,prion, alpha-beta transition
Title:
Low intensity ultrasound (LIUS) suppresses TGF beta induced hypertrophy in chondrogenesis of Adipose Stem Cells

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Abstract:
Introduction: Mesenchymal stem cells (MSC) are promising for cell-based cartilage regeneration. A yet unsolved problem is, the unwanted upregulation of hypertrophic markers like alkaline phosphatase (ALP) and collagen-type X during in vitro chondrogenesis and formation calcifying cartilage at transplantation sites. In contrast, mechanical forces has been shown is effective in development of joints and in vitro chondrogenesis as well. Therefore the aim of this study was to address whether addition of low intensity ultrasound to in vitro chondrogenesis protocol may inhibit undesired hypertrophy in differentiated MSCs. Materials and methods: Experimental groups of ASCs pellets (Ultrasound, TGFβ and Ultrasound/TGF) were cultured under chondrogenic condition (10ng/ml of TGFβ) and ultrasound conditions (200 mW/cm2; 10 min/day). After 2 week, differentiation was evaluated by histology, quantitative gene expression analysis and immunohistochemistry. Results: We found that LIUS prevents expression of Runx2II, collagen 10 and alkaline phosphatase genes in comparison with TGFβ. Alkaline phosphatase and Indian hedgehog (Ihh) immunostaining is prominently decreased in LIUS containing groups versus control groups. Conclusion: Our results indicate that low intensity ultrasound can suppress transient chondrocyte formation in general chondrogenic medium in presence and absence of TGFβ.

Keywords: Adipose tissue stem cells (ASCs), chondrocyte, low intensity ultrasound, TGFβ
Title: A new Strategy based on pharmacophore-based virtual screening in adenosine deaminase inhibitor s detection and in-vitro study

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Abstract:

Introduction: Adenosine deaminase (ADA) inhibition not only may be applied for the treatment of ischemic injury, hypertension, lymphomas and leukaemia, but also they have been considered as anti-inflammatory drugs. On the other hand according to literatures, ADA inhibitors without a nucleoside framework would improve pharmacokinetics and decrease toxicity. Hence we have carried out a rational pharmacophore design for non-nucleoside inhibitors filtration.

Methods: A merged pharmacophore model based on the most potent non-nucleoside inhibitor erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) and natural products were generated and applied for compounds filtration. The effects of filtrated compounds based on pharmacophore and docking studies investigated on ADA by UV and Fluorescence spectroscopy techniques.

Results: Extracted compounds were find efficiently inhibit ADA, and the most potent (2) shows an inhibition constant equal to 20 µM. Besides, Fluorescence spectroscopy studies revealed that enzyme 3D structure bear further change in lower concentrations of compound 2.

Conclusion: 3 non-nucleoside inhibitors for ADA are presented. According to obtained results from UV and fluorescence spectroscopy, such interesting pharmacophore template with multiple approaches will help us to extract or design compound with desired properties.

Keywords: Adenosine deaminase, Pharmacophore Docking, Lead discovery, inhibitor
Title:
Ionic Modified Epoxy Functionalized Polymer Gel as a High Performance support for Immobilization of Cellulase

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Abstract:
Epoxy-activated supports seem to be almost-ideal systems to develop very easy protocols for enzyme immobilization. Epoxy groups are very stable at neutral pH values and are able to react with different nucleophilic groups on the enzyme surface to form extremely strong linkages. However, epoxy groups are actually hardly reactive for enzyme immobilization under mild experimental conditions (neutral pH, low ionic strength). Herein we report a novel polymeric support which poly[(3-Trimethylammonium propyl methacrylamide chloride)-co-(glycidyl methacrylate)] is cross-linked by N,N'-methylenebisacrylamide to form an insoluble polymeric gel. Because of the ionic surface by reaction with epoxy groups on the ionic surface of gel, enzymes will absorb to the surface very fast and then covalently bonded to surface by reaction with epoxy groups on the surface of gel. To compare both nonionic and novel ionic-epoxy supports, we have evaluated their performance in the immobilization of cellulase and many parameters have investigated such as immobilization rate, immobilization yield, intrinsic activity of the immobilized derivative, and stability of stability of the final enzyme preparation.

Keywords: Epoxy, Immobilization, Enzyme, polymeric support
Title:
Molecular dynamics simulation of binding anti MS drugs (fingolimod and cladribine) to wild and mutated p53 gene for prediction of the carcinogen behavior it

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Abstract:
Two of the newest drugs that are proved effective on treating multiple sclerosis disease (MS) are cladribine and fingolimod. These drugs can be rarely carcinogenic. Due to aromatic ring, these drugs seem to bind to minor groove of DNA. Thus they may cause cancer by altering DNA structure or inhibition of transcription. Mutation rate in exon 8 of p53 gene is high and it can cause to more than fifty percent of cancers. Codon CGG is hot spot in exon8 which can changes to TGG in many cancers. The mechanism of binding of cladribine and fingolimod were investigated by docking method and molecular dynamics simulation methods. Then the binding free energy of these drugs to the wild and mutated forms of exon8 was calculated by LIE method. The results showed that the both drugs can bind to wild and mutated forms of exon 8 of p53 gene and change the structure of DNA. Then the mechanism of effect of these drugs probably is through binding to minor groove of DNA, they can inhibit transcription of p53 gene and cause to cancer. Also the results indicated that the binding free energy of fingolimod to mutated exon is more negative than wild exon and the binding of fingolimod to mutated exon is stronger than wild exon and it is more carcinogens. But binding of cladribine to wild exon8 is stronger than mutated form. Then Fingolimod is more carcinogens in mutation and cladribene is more carcinogens in wild P53 gene.

Keywords: cladribine, fingolimod, molecular dynamic, binding free energy
Title: Studies on the Interaction of a New Nickel Schiff base complex Containing N2O2 Donor Atoms with Calf Thymus DNA

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Abstract: Nucleic acids play an important role in biological systems and carry out a broad range of biological functions. In recent years, metal Schiff-base complexes have also found important applications in the biological field. Therefore, the interaction of these complexes with DNA has attracted much attention. In this work, the interaction between a new nickel complex containing tetradeinate Schiff base ligand with calf thymus DNA (CT-DNA) was measured by Viscometry method and UV-Vis spectrophotometry in physiological buffer (pH=7.4). The viscosity of DNA enhanced with increasing in the ratio of complex to DNA indicated the intercalative mode of binding. The midpoint of thermal transition (T_m) of DNA in the absence and presence of complex was measured. Data showed that by addition of complex, T_m of DNA increased, and confirmed type of complex intercalation. The results indicated that nickel Schiff base complex can bind to DNA and the major binding mode was intercalative binding.

Keywords: Calf-Thymus, DNA, schiff base complex, Interaction, Intercalation
Title: Cytotoxic activities of 2-benzylidene-6-(nitrobenzylidene)cyclohexanones against three human cancer cell lines

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Abstract: Background: Chalcones represent a class of cytotoxic compounds that are useful for cancer chemotherapy. The advantage of chalcones is the low propensity to interact with DNA; which decreases the risk of mutagenesity as the common side effect of current chemotherapeutic agents. A new series of compounds (2-benzylidene-6-(nitrobenzylidene) cyclohexanones) were synthesized with structural modifications of chalcones. In this study, we investigate the cytotoxic activities of these compounds against three cancer cell lines.

Methods: The dose dependent anticancer effects of these compounds were studied against SK-N-MC, MDA-MB-231 and K562 human cancer cell lines using MTT colorimetric assay. Etoposide was used as standard anticancer drug. The IC50 (50% inhibitory concentration) value were determined for each compound.

Results: All compounds with the exception of two showed significant cytotoxicity activity against tested cancer cell lines (IC50 values = 1.4-28.3 µg/ml). Most compounds displayed greater cytotoxicity than etoposide (IC50 = 21.9 µg/ml).

Conclusion: 3-(4-alkoxy-3-bromo-5-methoxybenzylidene)-4-chromanones compounds may be considered promising for the development of new anticancer agents.

Keywords: Cytotoxic agents, Cancer
Title: DNA binding Studies of some Palladium (II) Complexes of Amino Acide Derivatives by Ultraviolet-Visible Spectroscopic and Voltametric Methods

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Abstract: Studies in to the interactions between Palladium (II) Complexes and DNA are extremely important for drug discovery. The effect may be due to strong bonds of palladium with DNA which interact with it. In this study, we selected three structurally related Palladium (II) complexes of formula [Pd(Phen)(R-gly)]NO3 (where Phen is phenanthroline and R-gly is methyl, propyl and amyl-glycine). The interaction of ct-DNA with different concentration of these palladium (II) complexes were studied by ultraviolet-visible and voltametric measurements at 27 and 37 °C. These complexes have been interacted with DNA in Tris-HCl buffer solution containing 10 mM sodium chloride (pH=7.4) at 27 and 37 °C. the experimental results suggested that these complexes cooperatively bind to DNA presumably via intercalation. Moreover, the tendency of these Pd(II) complexes to interact with DNA was more in higher concentrations and temperatures than lower cases.

Keywords: Palladium (II) complexes, ct-DNA, Ultraviolet-visible, Voltumetric, Amino acid.
Title:
Efficient decreasing of the conformational entropy in a long lengths loop via nature of interactions in chondroitinase ABC I

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Abstract:
The conformational entropy has an important contribution to destabilization of the folded state of proteins; it may also affect the activity of enzyme. In this research, we investigated the effect of an interaction network which is established in the middle of a long length loop; located at C-terminal domain of chondroitinase ABC I. The three critical interactions of Asp689 with adjacent residues were changed via site directed mutagenesis and the kinetic parameters of single and combination of double mutants were assessed and compared with that of wild type enzyme. The variants were cloned in pET28a as expression vector. Maximum expression was reached at 27 °C, 0.7 mM IPTG in Luria-Bertani medium. After purification of enzymes with Ni-Sepharose column chromatography, the variants were then characterized. It was revealed that kinetic parameters of mutants increase relative to that of wild type enzyme. We concluded that increasing the flexibility of above-mentioned loop and its domain upon deletion of interactions results in improving the activity of enzyme; although it may have destabilizing effect on the structure of enzyme.

Keywords: Conformational entropy, interaction, site directed mutagenesis, kinetic parameters.
Title:
Heme degradation by interaction of hemoglobin with sodium dodecyl sulfate

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Abstract:
Introduction: Interaction of sodium dodecyl sulfate with hemoglobin has been investigated widely from different point of views. Two fluorescent products were found in the heme degradation reaction which has excitation wavelengths of 321 nm and 460 nm, respectively.

Methods: In this study the reaction of heme degradation of hemoglobin by sodium dodecyl sulfate has been studied using fluorescence spectroscopy and oxygen affinity methods.

Results: Results showed interaction of hemoglobin with sodium dodecyl sulfate produce heme degradation products. Addition of sodium dodecyl sulfate concentration lead to increase the fluorescence products to 1 mM sodium dodecyl sulfate. Functional studies showed that the low concentration of SDS leads to increase in oxygen affinity of hemoglobin and high concentration of sodium dodecyl sulfate leads to decrease in oxygen affinity of hemoglobin.

Conclusion: Our experimental results showed the reaction between hemoglobin and sodium dodecyl sulfate cause to heme degradation and changed function of hemoglobin.

Keywords: Hemoglobin, Heme degradation, Sodium dodecyl sulfate, Oxygen affinity.
Title:
Plasminogen Activator Inhibitor-1 gene polymorphism in Iranian Azeri Turkish patients with FMF disease and its association with amyloidosis

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Abstract:
Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by intermittent episodes of fever with serositis, arthritis or Erisiplemya. Plasminogen activator inhibitor 1 (PAI-1) is a key element in the inhibition of fibrinolysis by inactivating tissue-type and urokinase-type plasminogen activators. We evaluated the association of PAI-1 -675 4G/5G polymorphism with the severity of FMF disease. For this purpose, 89 FMF patients with M694V homozygous mutation and 95 healthy controls from Iranian Azeri Turks were selected. Detection of this polymorphism was performed by polymerase chain reaction using allele-specific primers. No significant association was found between patients and control group. However, These data showed that FMF patients with M694V homozygous mutation carrying 4G/4G genotype have a reduced risk for development of pleuritis (OR[odds ratios]= 0.36; 95%CI[confidence intervals]= 0.5-0.85; P value = 0.007) compared with 5G/5G homozygotes who have increased risk for development of amyloidosis (OR= 2.46; 95%CI= 1.29-4.72; P value = 0.001), pleuritis (OR= 2.55; 95%CI= 1.31-4.99; P value = 0.001) and fever (OR= 4.68; 95%CI= 2.04-10.96; P value = 0.000). Furthermore, the allelic frequency of the 4G among the patients with pleuritis was significantly low (OR= 0.5, 95%CI= 0.27-0.92, P value = 0.008). Conclusion: our data suggest a protective role for the 4G allele against pleuritis in FMF patients with M694V homozygous mutation in this cohort. More evaluation of this polymorphism may be important and require further studies.

Keywords: Familial Mediterranean fever, Plasminogen activator inhibitor 1 4G/5G Polymorphism, Amyloidosis, Iranian Azeri Turks
Title: 
Anti-proliferative mechanism of two novel Palladium complexes against Hela cancer cell line

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Abstract: 
Introduction: Palladium complexes are the first and most practical chemotherapy of cancer. Many Pd complexes have been used in chemotherapy treatments for testicular, ovarian, head and neck, bladder and lung cancers. Previous studies reported that Pd complexes demonstrated significant anti-tumor activity comparable with cisplatin. Further to that a new Pd complex demonstrating potent cytotoxic activity against different cancer cell lines has recently been reported. Better solubility of Pd complexes compared to Platinum, seems to make Pd complexes more attractive. In present study, we investigated the biological evaluations of two new designed Pd(II) complexes (1,10-phenanthroline butyl dithiocarbamato Palladium(II) nitrate and 1,10-phenanthroline hexyl dithiocarbamato Palladium(II) nitrate) via their anti-proliferative effects and death inducing mechanisms on model cancer cell line of Hela.

Methods: The cytotoxicity and anti-proliferative properties of Pd(II) complexes were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. In this study, various concentration of Pd(II) complexes (0 to 100 µM) were used to culture of the tumor cell lines for 24 and 48 h incubation times. Also, Doxorubicin (200 µg/ml) was as our positive control in cytotoxicity assays. In order to detect the mechanism of death inducing in Hela cancer cell line, the Flow cytometer kit of Annexin-PI was applied.

Results: The 50% cytotoxic concentrations (CC50) of the both complexes were determined 10 µM at 24 hours. Results show that the Pd(II) complexes produced a dose dependent response suppression on growing of Hela cancer cell line. Also, Flow cytometer results suggested that the anti-tumor activity of these complexes reveal typical morphology features of apoptotic death.

Keywords: Pd Complexes, Apoptosis, chemotherapy, Flow cytometry
Title:
Production and investigation on allergenicity properties of recombinant mutant β-Lg

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Abstract:
Introduction: Bovine β-lactoglobulin (β-Lg) is a globular protein contains 162 amino acid residues with molecular weight of 18.3 kDa. It belongs to the lipocalin family sharing similar structure and function. The biological function of β-Lg has not been fully known although this protein has an ability to bind many hydrophobic ligands such as retinol. β-Lg has been known as a major milk allergen. Many studies have been done in order to decrease its allergenicity properties. In the present study β-Lg was mutated on its major epitopes to decrease the allergenicity of this protein.

Method: The mutant plasmid was injected in yeast pichia pastoris and subsequently the production and purification of recombinant mutant protein has been done. The allergenicity properties of mutant β-lg was compared with native one using ELISA techniques.

Results: The binding of IgE from cow's milk allergy (CMA) patients to recombinant mutant β-LG using ELISA techniques showed that nearly 70 % of CMA patients sensitized to β-LG, it was observed that the mutation caused a decrease in its recognition by IgE.

Conclusions: It can be concluded that the mutation on the major epitopes of β-LG is associated with weaker binding of IgE from CMA patients to the mutated protein.

Keywords: β-lactoglobulin, Mutation, Allergy, ELISA techniques, Recombinant protein.
Title: Role of Epidemiological Investigation by IS6110-RFLP Method in Controlling New Transmission of Tuberculosis in Northwest of Iran.

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Abstract:

Introduction: In recent years in spite of medical advancement, tuberculosis remains as a worldwide health problem. Identifying the source of transmission of infection is necessary for decreasing of tuberculosis (TB). Determining the variety of TB strains by DNA fingerprinting can help for this aim. In this study two separate studies were performed in different duration of time and compared for revealing changes in manner of transmission of tuberculosis in Northwest of Iran.

Method: The study population comprised all patients from whom at least one sample was positive for M. tuberculosis by culture which was collected in four TB centers of the province, for the first study from September 2002 to March 2003, and second study from March 2004 to March 2005. Samples were analyzed by IS6110 restriction fragment length polymorphism. Isolates having identical RFLP patterns were considered a cluster.

Results: In the first study twelve clusters were found among total of 38 strains. The clusters included 26 patients that were identified by 12 others. 93 distinct IS6110 patterns were revealed. Eighty-one of these patterns were unique and 12 were shared by 2 to 8 strains. The minimum estimate for transmission in first study was 22%. In second study 123 different patterns were observed. 16 of these were shared by two or more patient’s isolates and were detected in 47 strains (clustered isolates). The remaining 107 patterns were found only once (unique isolates). Transmission rate of second study was 23.91%.

Conclusion: These studies showed a low increasing of transmission rate of tuberculosis in Northwest of Iran. On the other hand there was a high decrease of transmission of tuberculosis with foreign source and in the second study there was no report of clusters accompanying.

Keywords: Keywords: Tuberculosis, Transmission, IS6110, Fingerprinting.
Title:
Non-Enzymatic Glycation Protects Calcium Induced total lens soluble Proteins Aggregation

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Abstract:
The vertebrate eye lens comprises three major proteins such as α-, β- and γ-crystallin (Cry). While α-Cry belongs to chaperone super family, β- and γ-Cry play a structural function in the lenticular tissue. The correct assembling of lens crystallins affect by both environmental and physiological factors which are important for maintenance of lens transparency. Since both hypo- and hypercalcemic conditions are involved in lens cataractogenesis, calcium homeostasis is important for lens transparency, and opacification. The normal range of calcium concentrations in bovine eye lens is about 0.5 to 2 mM, however in some cataract eye lens calcium level is increased to 13 folds of its normal concentration. In this study total soluble lens proteins were subjected to the extensive non-enzymatic glycation for one month at 37 °C. The extent of glycation was confirmed by both o-phthalaldehyde (OPA) and fluorescamine assay. Both glycated and non-glycated TSP samples were incubated in the presence of calcium (0.5-80 mM). The aggregation/fibrillation propensity of these TSP samples was compared by gel electrophoresis and different spectroscopic methods. While non-glycated TSP readily becomes turbid, the glycated protein counterpart resists against aggregation. Overall, this study suggests that non-enzymatic glycation could protect TSP against calcium induced aggregation and this modification can be relevant to the stability of these proteins which are important for the protein turnover and their cataractogenesis during aging and diabetes.

Keywords: Total soluble lens protein (TSP), Glycation, Aggregation, Calcium, Stability.
Title:
In silico analysis to predict how ZFX gene exerts its antiapoptotic function

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Abstract:
Introduction: Located on the X chromosome, ZFX gene is structurally similar to a related gene
on the Y chromosome. It encodes a member of the krueppel C2H2-type zinc-finger protein
family. Studies in mouse embryonic and adult hematopoietic stem cells showed that this gene is
a shared transcriptional regulator for self-renewal of both embryonic stem cells (ESCs) and
hematopoietic stem cells (HSCs). Knock down studies in different cancerous cell lines shows
that this gene is critical for cell survival. Due to this, we hypothesize that this gene may affect
the expression of some antiapoptotic genes including Bcl-2, Mcl-1, and Survivin.

Method: The promoter sequence of the mentioned antiapoptotic genes was defined using
promoter prediction neural network tool and Aceview. Then the sequence used as an input in
the zinc finger tools server to search the sequence for zing finger protein target sites.
Furthermore, we searched ZFX recognition sequence as defined for HLA-A11 in the upstream
sequence of the above mentioned genes.

Results: The output results of the zinc finger tools server showed that there is recognition
sequence for the zinc finger proteins in the upstream region of the above mentioned genes.
Furthermore, we found the ZFX recognition sequence in the upstream sequence of Bcl-2 and
Mcl-1 genes.

Conclusions: According to our results, it is possible that the ZFX protein exerts its antiapoptotic
function by upregulating Bcl-2 and Mcl-1 genes. Further investigation should be done to clarify
the precise molecular mechanism of this action.

Keywords: Cancer, ZFX gene, Antiapoptotic genes, In silico
Title: synthesis of PCL nanofiber with different roughness & plasma modification

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Abstract: the field of tissue engineering is an emerging discipline that applies basic principles of life sciences and engineering for the repair and restoration of human tissues and organs. in this study, scaffolds were fabricated by poly(ε-caprolacton) (PCL) through electron spinning technique. these scaffolds have various values of nano-roughness and plasma surface modification.

the cell-biomaterial interactions were studied by culcuring human fibroblast cells on nanofiber PCL. the features of nano-PCL surfaces were characterized using an atomic force microscopy (AFM) to observe the topography.

we expect that nano-scale scaffolds with various values roughness and plasma surface modification, can provide the optimal conditions for the cellular attachment and proliferation.

Keywords: tissue engineering, roughness, nanofiber, scaffolds, plasma modification.
Title:
The study of p53 polymorphism at codon 72 in patients with thyroid cancer in East Azerbaijan province

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Abstract:
Introduction: Thyroid cancer is the most common malignancy of the endocrine system. Ethnic and geographic discrepancies are involved in cancers. Cancer associated with mutation in tumor suppressor genes and oncogene. In addition to mutation, genetic polymorphisms are involved in incidence of cancer. Purpose: The purpose of this investigation was studying polymorphism at codon 72 of p53 gene in patients with thyroid cancer in East Azerbaijan province. Material and Methods: In case-control study 60 patients with thyroid cancer in different age and sex groups, as well as 99 healthy individuals as controls were collected in East Azerbaijan. After extraction of DNAs from these people bloods by Proteinase K protocol, different genotypes of 72th codon of p53 gene were determined by amplification refractory mutation system (ARMS) and presence of probable mutation, single strand conformation polymorphism (SSCP) technique was conducted. Also for confirm the results, Exon 4 of p53 gene was investigated by DNA sequencing. Result: In the control group, the genotype distribution of p53 polymorphism for Arg/Arg, Arg/Pro and Pro/Pro genotypes was 30.3%, 50.5% and 19.2% respectively. In the caner groups, the distribution was 31.67%, 51.67%, 16.67%, respectively for the same order of genotypes. Discussion: This study indicates that polymorphism at codon 72 p53 gene is not a genetic predisposing factor for thyroid cancer in this population due to from ethnic, geographic discrepancies and p53 mutation.

Keywords: thyroid cancer, p53 gene, codon 72, polymorphism
Title:
Recombinant lysostaphin; a novel approach for treatment of staphylococcal infections

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Abstract:
Objective: Over the last decade, a dramatic increase in the prevalence of antibiotic resistance in several medically significant bacterial species has been seen. There is an urgent need to develop novel antibacterial agents to eliminate multidrug-resistant bacteria. A very interesting class of novel antibacterials are enzybiotics consist of lysins, bacteriocins, autolysins, and lysozymes. Bacteriocins are peptides or proteins produced by bacteria to inhibit the growth of other bacteria. The bacteriocin whose antibacterial activity has been studied most thoroughly is Lysostaphin, an endopeptidase encoded by Staphylococcus simulans that specifically cleaves glycyl-glycyl bonds in the interpeptide cross-bridges of the staphylococcal peptidoglycan and is very efficient in lysing S. aureus and can kill practically all strains of this species, including MRSA, NRSA and strains with reduced susceptibility to vancomycin, also it has other medical applications such as: elimination of staphylococci colonizing nasal mucous membrane, prevention of catheter colonization by enzyme molecules coating their surface, and the treatment of staphylococcal infections. Since further evaluation of the anti-staphylococcal potential of lysostaphin as a therapeutic agent depends on the availability of large amounts of highly purified protein from a safe-nonpathogenic source, we can construct a recombinant plasmid which overproduces mature lysostaphin in the cytoplasm of E. coli cells transformed with the recombinant plasmid. Conclusion: The cloning, expression, and purification procedure of recombinant lysostaphin from a non-pathogenic organism; E. coli; enables preparation of large quantity of r-lysostaphin for structure-function studies and evaluation of its clinical potential in therapy and prophylaxis of staphylococcal infections.

Keywords: Lysostaphin; Staphylococcus simulans; antibiotic resistance; Recombinant
Title: Preparation and in vitro evaluation of doxorubicin loaded magnetite nanoparticles modified with PLGA-PEG4000 copolymers

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Abstract: Superparamagnetic iron oxide nanoparticles are attractive materials that have been widely used in medicine for drug delivery, diagnostic imaging, and therapeutic applications. In our study, superparamagnetic iron oxide nanoparticles and the anticancer drug, doxorubicin hydrochloride, were encapsulated into poly (D, L-lactic-co-glycolic acid) poly (ethylene glycol)(PLGA-PEG) nanoparticles for local treatment. The magnetic properties conferred by superparamagnetic iron oxide nanoparticles could help to maintain the nanoparticles in the joint with an external magnet.

Methods: PLGA:PEG triblock copolymer was synthesized by ring-opening polymerization of D, L-lactide and glycolide, and PEG4000 as an initiator. The bulk properties of this copolymer was characterized using ¹H nuclear magnetic resonance spectroscopy, gel permeation chromatography, Fourier transform infrared spectroscopy, and differential scanning calorimetry. In addition, the resulting particles were characterized by x-ray powder diffraction, scanning electron microscopy, and vibrating sample magnetometry.

Results: The doxorubicin encapsulation amount was increased to a great extent for PLGA:PEG4000 triblock copolymer. This is due to the increased water uptake capacity of the blended triblock copolymer, which encapsulated more doxorubicin molecules into a swollen copolymer matrix. The drug encapsulation efficiency achieved for Fe₃O₄ magnetic nanoparticles modified with PLGA:PEG4000 copolymer 78% and the release kinetic was controlled. The in vitro cytotoxicity test showed that the Fe₃O₄-PLGA:PEG4000 magnetic nanoparticles had no cytotoxicity and were biocompatible.

Conclusion: There is potential for use of these nanoparticles for biomedical application. Future work includes in vivo investigation of the targeting capability and effectiveness of these nanoparticles in the treatment of lung cancer.


Keywords: keywords: superparamagnetic iron oxide nanoparticles, triblock copolymer, doxorubicin encapsulation, water uptake, drug encapsulation efficiency.
Title:
In Silico investigation on the function heterologous signal peptide on the expression of recombinant Human F9 in mammalian cell line

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Abstract:
Introduction Clotting factor IX (F IX) is avitamin K dependent plasma glycoprotein that plays a crucial role in blood coagulation. Reduced levels or a missing or dysfunctional FIX glycoprotein are associated with a bleeding disorder called hemophilia B. Human plasma–derived factor IX (pdFIX) concentrates are routinely used to treat patients, but concerns remain regarding transmission of blood-borne pathogens. Therefore, the safety and efficacy of recombinant human factor IX (rFIX) were evaluated.

Eukaryotic proteins generally have embedded signals, such as secretory signal peptides (SP). Besides, application of suitable signal peptide is crucial for both secretion efficiency and expression level of FIX in heterologous expression systems. The signal peptide is at the N-terminus of the secreted protein and includes the start methionine, a positively charged N-region, a central characteristic hydrophobic region, and a C-region ending with a cleavage site. Several programs have been applied to predict signal peptides, for example, lexical definition, weighted profiles, PSORT, PHYSEAN, SignalP, and PrediSi with varying degrees of accuracies.

Materials and Methods
In this study we investigated the function of signal peptides on the expression of FIX comparatively. With this aim, we used SignalP, and PrediSi programs for prediction and evaluation of the examined signal peptides. Subsequently, the obtained results were examined in vivo. Using molecular techniques, we amplified and joined the signal peptide coding region to the hFIX cDNA, correspond to the mature proprotein. The chimeric fragment was examined for transient expression of the hFIX in mammalian cell line in comparison with both the hFIX native signal peptide, under a CMV regulation. The expression efficiencies of hFIX expressed by the recombinant cells was analyzed by RT-PCR, ELISA and coagulation test.

Results and Discussion
Using the neural network-based prediction programs, we evaluated the scores for cleavage position and secretion efficiency of the Human prothrombin signal peptide, among the several examined signal peptides. According to the results obtained the Human prothrombin signal peptide was predicted as a suitable signal peptide in comparison with that of the hFIX. Our primary results showed that substitution of hFIX native prepeptide sequence with that of the Human prothrombin might be worth trying to be examined in further stable expression analysis.

Keywords: Human factor 9, SignalP, PrediSi, Prothrombin
Title:
Role of Galectin-3 on survival and cell cycle human ovarian cancer cell line SKOV-3

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Abstract:

Introduction: SKOV-3 ovarian cancer cell line expresses galectin-3 and is Paclitaxel (Pac.) resistant. Galectin-3 (Gal-3) a carbohydrate-binding protein which is involved in cancer cell growth, adhesion and invasion. The aim of present study is to assess combined cytotoxic effect of Pac and Pectasol (inhibitor of Gal-3) on SKOV-3 cell line. Methods: Cells were treated with Pac (10, 50, 100, 250, 500 and 1000nM) and/or Pectasol (0.025, 0.05 and 0.1%) for 24 and 48 hours. Cell viability was determined by using MTT assay, apoptosis was revealed with caspase-3 colorimetric assay and cell cycle was analysed by flowcytometry method. For combination study 100 nM Pac and 0.1% Pectasol were used.

Results: Pectasol alone led to 19% decrease of cell survival regardless of its concentration. The maximum effect of Pac was observed at 1uM which showed 35% decreased cell survival. However when combined with Pectasol there was a significant reduced cell viability by 60% and 75% compared to control after 24h and 48h, respectively (P<0.001). Pectasol alone did not affect apoptosis but induced G1 arrest. Pac alone led to maximum 5% cells in subG1 after 48h. However combination of Pectasol and Pac showed significant increase of apoptotic cells (15% versus 1% in control 24h, P<0.05 and 35.67% versus 0.82% in control, P<0.001 after 24h and 48h, respectively) In parallel, caspase-3 activity was significantly increased by 1.9-fold and 3.8 fold compared to control (P<0.001).

Conclusion: Our results suggest that inhibition of Gal-3 could be a useful therapeutic tool for combination therapy in ovarian cancer.

Keywords: Galectin-3, Pectasol, Paclitaxel, Combination therapy, Ovarian cancer
Title: Wnt11 affects survival and adhesion of human ovarian cancer cell line SKOV3

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Abstract:

Introduction: Ovarian cancer is the most common and lethal cancer in women. Previous reports demonstrated alteration of Wnt signaling in ovarian cancer as Wnt11 was shown to be expressed in ovarian cancer cell lines. However, the role of Wnt11 in epithelial ovarian cancer remains unknown. In this study, we sought to determine the role of Wnt11 on survival, and adhesion of ovarian cancer Skov3 cell line by using SiRNA Wnt11.

Methods: Skov3 cell line was transfected with SiRNA Wnt11 with lipofectamine 2000 or SiPORT as transfection reagent. After 48 hours post-transfection Wnt11 expression was assessed with western blot. In parallel, survival/proliferation of transfected cells was assessed by using MTT test. Adhesion test was performed after 48 h post-transfection with SiRNA Wnt11 in coated or uncoated wells with thin layer of matrigel.

Results: Our results showed significant increased cell survival in transfected cells compared to control in the absence of serum (147% versus 100% in control). Although not significant, similar effect was observed in the presence of serum. In addition, decreased Wnt11 expression by 50% with 150 nM SiRNA Wnt11 was observed as revealed by western blot. Adhesion of transfected cells was increased compared to control after 15 minutes post seeding in coated wells with thin layer of matrigel (63% versus 37% in control).

Conclusion: These data may suggest a significant role of Wnt11 in survival, and adhesion in ovarian cancer which needs further molecular investigation.

Keywords: Ovarian cancer, Wnt11, SKOV3, Cell survival, Adhesion
Title: 
The Effect of nitrogen on the cytokinin accumulation in tobacco (Nicotiana rustica L.) under in vitro culture

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Abstract: 
Nitrogen (either nitrate or ammonium) is one of the microelements that has a major role in plant growth and metabolism. Besides, it plays a vital role in Adenin (Aminopurine) biosynthesis, which can convert to cytokinins. Therefore, studying the effect of this mineral element on the synthesis of Zeatin, which controls cell division and plant growth seems promising. To this end, plantlets grown from tobacco seeds were transferred to the MS media containing nitrogen with three concentrations (0.1M, 0.2M, 0.4M) and an nitrogen free medium as control. For purification of cytokinin method of Unyaryar et al. (1996) was applied. The level of cytokinin was measured using spectrophotometer. Results showed the highest level of Zeatin in 0.4M concentration of nitrate and the lowest level in free nitrogen media. No significant difference was observed between the concentrations of 0.1M and 0.2M. The total chlorophyll in concentrations of 0.1M and 0.2M nitrogen was significantly more than the concentration of 0.4M and the control. Moreover, carotenoid in 0.1M and 0.2M nitrogen was higher than concentration of 0.4M. It can be concluded that the increase in the concentration of nitrogen of the medium resulted in the higher accumulation of Zeatin in tobacco plants.

Keywords: Cytokinin, Zeatin, Nitrogen, Tobacco, tissue culture.
Title:
MDR1 C3435T polymorphism associated with the development of clinical features in Behcet’s disease in Iranian Azeri Turkish patients.

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Abstract:
Behcet disease (BD) is a systemic vasculitis of unknown cause with higher prevalence along the ancient Silk Road. Behcet’s occasional familial aggregation and its close association with genes of major histocompatibility complexes justify that genetic factors play an important role in the development of the disease. In this study, we evaluated the association of Multi Drug Resistance (MDR1) C3435T polymorphism with the severity of BD disease. For this purpose, we investigated the distribution of MDR1 C3435T polymorphism in 69 BD patients of Iranian Azeri Turks and 92 ethnically sex-matched healthy controls, via the PCR–RFLP technique. Although there was no significant association of MDR1 C3435T polymorphism between two groups of patients and healthy controls, our data showed substantial association of CC genotype with the development of several clinical features. These results suggest that, CC genotype is a risk factor for development of some clinical features of BD in patients from Iranian Azeri Turk ethnic group.

Keywords: MDR1. Polymorphism. C3435T. Pgp. Iranian Azeri Turks.
Title:
Production of a single chain variable fragment of anti human CD4 receptor antibody fused to green fluorescent protein in Escherichia coli

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Abstract:
Introduction: Antibodies are powerful tools to study protein functions, protein localization and protein-protein interactions. Antibodies are usually conjugated to fluorescent molecules for research and diagnostic purposes which are termed fluobody.
In this study, an anti-human CD4 single-chain antibody fragment (scFV) was cloned and genetically linked to the C terminus of the enhanced green fluorescent protein (EGFP) at cDNA level to generate an EGFP/scFV fusion protein. Different sets of expression vectors were constructed that permitted the efficient fusion and expression of CD4 scFV to EGFP. The effects of different temperatures were also assessed in expression of the fusion protein and fluorescent emission of EGFP.
Method: Different vectors including pAB1 and pCANTAB5E were used to clone the CD4 scFV EGFP. Escherichia coli strain, Origami, were transformed by these vectors and the level of expression in four different temperature was assessed using flow cytometry, fluorescent microscopy, SDS PAGE, dot blot and western blotting.
Results: Several constructs of fluobody containing EGFP and CD4 scFV were cloned and expressed in E.coli. In lower temperatures and in pCANTAB5E vector, expression of fluobody was higher than those conditions with high culture temperatures and pAB1 vector.
Conclusions: The present recombinant fluobody is a bifunctional antibody which retains both antigen binding activity and its fluorescence simultaneously. Thus lower temperature culture condition for high production of this fluobody seems to be required for appropriate purification of this fluobody for therapeutic and diagnostic applications.

Keywords: fluobody, green fluorescent protein, single-chain antibody fragment(scFV), Escherichia coli.
Title: In vitro evaluation of the antiadhesion properties of probiotics against caries pathogens

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Abstract: Introduction: Probiotics are viable microorganisms which improve human health by reducing or inhibiting the number of pathogenic microorganisms. The use of probiotics to improve oral health is highly regarded. The aim of this study is investigating the antiadhesion properties of probiotics on caries pathogens.

Method: In this study four strains of lactobacilli (L. casei, L. paracasei, L. reuteri, L. plantarum) as probiotics and two strains of streptococcus (S. mutans, S. sobrinus) were used as caries pathogens. Their adherence and biofilm formation were initially investigated by microtitre plate assay and then the effect of probiotics on adhesion of pathogens was evaluated using microtitre plate assay. Results: S. mutans and S. sobrinus indicated strong and intermediate adherence respectively. All the lactobacilli were significantly reduced the adhesion of pathogens. The effectiveness of lactobacilli can be explain this way: L. plantarum > L. casei > L. paracasei > L. reuteri. It should be noted that the lactobacilli were mostly reduced the adhesion of S. mutans. Conclusions: Observations showed that probiotics had antiadhesion effect in the caries pathogens.

Keywords: Probiotic, Streptococcus mutans, Streptococcus sobrinus, biofilm, antiadhesion activity.
Title:
Cloning and sequencing of Cystatin C (CST3) gene from Human's Tissue in E.coli

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Abstract:
Background: Cystatines have regulative and preventive roles of Cysteine Proteases presenting in the every biologic fluids of the human body. For human, the coding gene of this protein located in chromosome 20 and about mice, on the chromosome 2. The target of the present study is cloning and sequencing of cystatine C gene from the healthy human tissue.

Methods: In this research when total RNA extracted from human cord, thyroid, breast and blood tissues, cDNA was synthesized by RT-PCR method. afterwards, two restriction site for BamH I and Hind III enzymes was distinct over the sequence of cystatine C gene and after digesting by those enzyme, the gene was insert to the plasmid pET28a as a vector by T4 DNA Ligase, then cloned in E.coli DE3-BL21 as a porter receiver.

Result: Segment that had cloned in the bacteria was amplified and DNA plasmid was extracted by miniprep technique then it was cut by enzyme and enzymatic chain reaction was done by specific primer, finally, favorite product in order to be sequenced was send to the foreign company.

Conclusion: After checking the results and surveying the sequence we compare it with the bioinformatic software's, The thing that observed just was a deletion in the place of primer that gave us a 99% alignment also any track of pathogenic alleles did not observed.

Keywords: Anti-protease, CST3, cysteine proteases, human chromosome 20, Gene cloning
Title:
Interaction between (E)-3-(3-(2,3-dimethoxyphenyl) acryloyl)-6-hydroxy-2H-chromen-2-one (DAC) with human serum albumin

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Abstract:
Human serum albumin (HSA), the major carrier of blood components, is the most abundant plasmaprotein of the globular proteins family with a complete alpha-helical structure. HSA is able to transfer a lot of drugs such as coumarin. Coumarin is found naturally in many plants, which has a low toxical effect to the human body. (E)-3-(3-(2,3-dimethoxyphenyl)acryloyl)-6-hydroxy-2H-chromen-2-one (DAC), as a new derivative of coumarin, has anti-cancer effects comparable with cis-platin and much lower toxicity. This study was focused on the interaction between DAC and HSA as a drug carrier. Fluorescence spectroscopy were carried out in aqueous solutions at two temperatures of 25 and 37 ºC and were complemented with molecular docking simulation for molecular details. Spectroscopic analysis of the emission quenching has revealed that the quenching mechanism of DAC is a static quenching mechanism. Using the modified Stern–Volmer equation, the number of binding sites was determined close to 1 at both temperatures of 25 and 37 ºC. Since HSA has many potential binding sites, a blind docking was conducted over the protein after dividing it to eight overlapping boxes of equal size. The results were then refined via re-docking with finer grids of interactions. Reasonable agreement was found between computational and experimental results. This study can be useful in rational drug design to have fewer side effects for drugs.

Keywords: Human serum albumin, Drug binding, Coumarin, Fluorescence
Title: Cytotoxicity evaluation of new synthesized Pd(FIP)2 complex against K562

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Abstract: Medicinal inorganic chemistry is a developing research area that began in 1962 with the synthesis of cis-diamminedichloroplatinum (II), later known as cisplatin. It is mainly used in the treatment of several types of cancer, including ovarian, head and neck, bladder, cervical and lymphoma. Since the activity of palladium complexes have generally been shown to be lower than that of platinum analogs with similar structures, many palladium (II) and palladium (I) neutral complexes were found to exhibit antitumor activity.

Then, in present investigation, we have synthesized and characterized a new designed Pd(FIP)2 complex (where FIP is (2-foran-2-yl) 1H-imidazo-[ 4,5-f] (1,10-phenanthroline). Also, the cytotoxicity and growth inhibitory effects of the complex were determined on the human leukemia cell line of K562, (chronic myelogenous leukemia), as cancer model cell line. The cytotoxicity and anti-proliferative properties of Pd(II) complex was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. In this study, various concentrations (M) were used to culture of the tumor cell of Pd(II) complex (0 to 120 lines after 24 h incubation time. The 50% cytotoxic concentration (Cc50) of this complex was determined 95 µM. Results show that the Pd(II) complex produced a dose and time – response suppression on growing of K562 leukemia cell lines.

Keywords: Pd(II) complex, cytotoxicity, cancer, cis platin
Title: 
Investigation of Conserved Electrostatic Interactions in the Nucleosome Structure

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Abstract:

Introduction: DNA-protein specific interactions fall into two categories: the base readout and the shape readout mechanisms. Base readout includes direct determination of DNA bases by direct hydrogen bonding between DNA bases and amino acid side chains. In shape readout, with smaller affinity, the DNA bases are recognized based on the DNA local geometry and deformability. The nucleosome formation dependence on the nucleotide sequence is an example of a shape readout mechanism. While nucleosome mapping of a variety of Eukaryotic genomes have been established, it is still not clear how this positioning dependency occurs. It is generally accepted that patterns of hydrogen bindings are mainly responsible for positioning of the DNA along the nucleosome.

Method: We have investigated the electrostatic interactions importance by sorting the most prominent and conserved electrostatic interactions in histone-DNA complexes. Three positively charged amino-acids namely Arginine, Lysine, and Histidine have been considered, especially those in direct contact with DNA bases. We have found considerable similarity of amino acid-nucleotide contacts in different nucleosome structures (Using seven PDBs of nucleosome structures). To quantify the electrostatic interactions in nucleosome complex we utilize the APBS software.

Results: We have found three different patterns for arginine residues in direct contact with DNA nucleotides. One important conserved pattern includes 14 arginins inserted deeply into minor grooves.

Conclusions: Electrostatics interactions, hydrogen bonding, hydrophobic interactions and water mediated hydrogen bindings are the four interactions in nucleosome structure between DNA and histones. Our results indicate that the electrostatic interactions have a key role in nucleosome structures and sequence dependency.

Keywords: DNA-Protein specific interaction, Nucleosome, Electrostatic.
Title:
Antibacterial activity of two new Nickel and Cobalt Complexes

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Abstract:
Industrial production and the use of metal transitions can cause environmental pollution. On the other hand, some of these metals are present in trace amounts as essential elements for biological systems and these metal ions also play an important role in bioinorganic chemistry. In order to understand the role of these metal ions in biological systems, structural studies of the biological compounds and their metal complexes are extremely important. The antibacterial activity of two Ni(FIP)2(ClO4)2 and [Co(TIP)2](CH3COO)2 complexes (where FIP is (2-foran-2-yl)1H-imidazo-[4,5-f](1,10-phenanthroline) and TIP is (2-thiofen-2-yl)1H-imidazo-[4,5-f](1,10-phenanthroline)) were tested on Gram positive bacteria, Staphylococcus aureus and Bacillus cereus, and Gram negative, E. coli. Antibacterial activities were estimated on the basis of the size of the zone of inhibition formed around the paper disks on the seeded agar plates. The results were obtained that the biological activity of the Co(II) complex was equal to Ni(II) complex biological activity was increased with less concentration.

Keywords: Antibacterial activity, Nickel Complex, Cobalt complex
Title:
Study on the specific activity of mitochondrial membrane-bound enzymes upon interaction with lysozyme amyloid aggregates

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Abstract:
Recent findings implicate that the protein aggregates induce neurotoxicity predominantly in their intermediate oligomeric state. Although there appears to be a consensus on membrane permeabilization by amyloid oligomers, studies on the effects of oligomers on the activity of membrane-bound enzymes are rare. Therefore, in the present report, we describe the effects of the monomer, oligomer and fibrils of hen egg white lysozyme (HEWL) on the activity of two mitochondrial membrane-bound enzymes including rotenone-insensitive-NADH-cytochrome c reductase and β-hydroxybutyrate dehydrogenase, as marker enzymes for mitochondrial outer and inner membranes, respectively. A variety of techniques, including thioflavin T and ANS binding assays, fluorescence quenching, transmission electron microscopy and circular dichroism, were employed to characterize the toxic structures. HEWL oligomers were found to be flexible and hydrophobic structures with the capacity to interact with mitochondrial membranes. The specific activity of the two enzymes was markedly reduced upon exposure to HEWL oligomers, but not native monomer or mature fibrils. Moreover, the oligomer–induced decrease in the specific activities was influenced in the presence of calcium and spermine. It is concluded that the changes in lipid-protein-interactions of mitochondrial membranes, induced by oligomer species, may be affected the activity of membrane enzymes.

Keywords: Hen egg white lysozyme, mitochondria, cytotoxicity, oligomer
Title:
Synthesis and characterization of water borne biodegradable poly(urea-urethane) anionomers based on L-leucine cyclodipeptide (LC) and PEG: study of their degradability in activated Sludge

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Abstract:
After almost half a century of application of polyurethanes in the health field, they remain one of the most popular group of biomaterials applied for medical devices. During the next decade PUs became extensively researched for their relative sensitivity to biodegradation and the desire to further understand the biological mechanisms in biodegradation tests. On the other hand the emission of organic solvent is a severe problem for solvent-borne polyurethanes. It is mandatory to decrease the level of volatile organic compounds for environmental and health protection. Polyurethane dispersions have received increased attention because they are non-toxic, non flammable and do not pollute the environment. An active sludge, composed of the living microorganisms such as bacteria, fungi and protozoa that act in the metabolism of the influent organic material. Depending on the operational conditions, more complex organisms like ciliates and rotifers may also be present. In this work we prepared L-leucine cyclodipeptide (LC). Then a new class of degradable water-borne poly(ether-urethane-urea)s (PEUUs) was synthesized via the reaction of the 4,4’-methylene-bis-(4-phenyl-isocyanat), LC and polyethylene glycols (PEG) with molecular weight (MW) 1000 and dimethylol propionic acid (DMTA), as internal emulsifier. Some degradation characteristics of these polymers in activated sludge before and after degradation was studied via FTIR, TGA and SEM techniques. It was shown that hard segment degraded selectively as much as soft segment and these water borne PEUUs are susceptible to degradation in sludge much more than solvent borne PEUUs which were not containing DMTA group.

Keywords: Biodegradable polyurethane, water borne polyurethane, sludge biological test
Title: Synthesis of biodegradable water dispersion poly (urea–urethane) based on L-leucine anhydride (LA) and polyethylene glycol: study of polymer behaviour in bacterial environment

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Abstract: During the past two decades significant advances have been made in the development of biodegradable polymeric materials for biomedical applications. Biodegradation of polymeric biomaterials involves cleavage of hydrolytically or enzymatically sensitive bonds in the polymer leading to its erosion. Polyurethane ionomer dispersions were prepared with ionic groups in soft segments. Ionic soft segments gave significantly lower solution viscosity, smaller particle size and greater dispersion viscosity compared with ionic hard segments. Water dispersion poly (urea–urethane)s because of proper degradation properties and low toxicity rather than solvent-borne polyurethanes were more interested. In contrast to fungi which are aerobic in polymer degradation Bacteria can either be aerobic or anaerobic. Their degradative action is also chiefly a result of enzyme production and resultant break-down of the nonliving substrate in order to obtain nutrient materials. In this work we prepared L-leucine anhydride (LA) as peptide base monomer. Then a new class of degradable water dispersion poly (urea–urethane) was synthesized via the reaction of the LA, 4,4′-methylene-bis-(4-phenyl-isocyanat) and polyethylene glycol and dimethylol propionic acid (DMTA), as anionic dispersing agent. Then we studied the degradation of polymer in bacterial environment and investigated its nutrition effect. Some degradation characteristics of this polymers in the presence of Bacillus bacteria was followed via SEM, TGA and FTIR techniques. A significant rate of degradation was observed in PUU samples under bacterial environment. The PUU showed nontoxic effect to bacteria. Biofilm formation on polymer surface supports its role as nutrition source.

Keywords: Biodegradable polyurethane, water borne polyurethane, bacterial test
Title:
Effects of Silica Nanoparticle Ionic Liquid as a Green Additive on Thermal Reversibility of Horse Liver Alcohol Dehydrogenase

Authors:
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Abstract:
Introduction: Horse Liver alcohol dehydrogenase (HLADH) is a dimer enzyme, playing role in alcohol to aldehyde/ketones conversion in the body, also is a commercial biocatalyst. Thus the research on the stability of this biocatalyst is a great major for helping industry. Ionic liquids (ILs) have recently emerged as a replacement for conventional organic solvents for a wide variety of synthetic reactions due to their excellences including negligible vapor pressure, high thermal and chemical stability, recoverability and recyclability. In this work the stability and the thermal reversibility of HLADH was studied in the presence of silica nanoparticle supported imidazolium ionic liquid. Method: Calorimetric study was carried out by nano-DSC differential scanning calorimeter. The protein and nano-ionic liquid concentration was 0.6 mg/ml and 0.1 mg/ml. Results: Denaturation of HLADH at elevated temperatures was studied by DSC. The thermal denaturation of this enzyme proved to be fully irreversible, and the heat absorption curve was represented by the main thermal transition at 64.6 °C. Here, the silica nanoparticle supported imidazolium ionic liquid was utilized as a green additive for studying the thermal reversibility of HLADH. The reversibility was enhanced 36% and the Tm was increased 1 °C by adding this nano-ionic liquid.

Keywords: Horse liver alcohol dehydrogenase, thermal reversibility, ionic liquid
Title:
Callus tissue induction for extraction of a cycloalkane derivative from Apium graveolens

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Abstract:
Introduction: Nowadays, isolation of useful natural products from plant callus culture has been used as a modern biotechnological technique to obtain those useful chemicals. *Apium graveolens* (Apiaceae) is a well known edible herb was known to produce a variety of plant secondary metabolites such as flavonoids and coumarins. In this study, we focused on induction of callus tissue of the plant seedling that may be used as a source of different metabolites.

Method: The seeds of the plants after sterilizing were cultured in a petri dishes line with MS medium. After emergence of seedling, radicle segments were transferred to another MS cultures with contain different combination of plant hormones, *kin* and *2,4-D*. The petri dished incubated in a growth chamber at 25°C and certain photoperiod. Weight of produced callus were measured for all treatments. On the other hand, n-hexan extract of dried callus was obtained by a soxhelet apparatus and were analyzed using thin layer chromatography technique (TLC) to afford a colorless oily substance. The structure of isolated compound was elucidated by spectroscopic methods such as IR, UV, Mass and 13C and 1H NMR.

Results: Our results indicated that although callus induction take place in MS medium without phyto hormones, maximum callus production induced at MS medium with 2, 4-D (4 mg/L) and kinetin (2mg/L). The structure of isolated natural product from the callus was determined as cyclodecane.

Conclusions: It was be concluded that callus tissue of *Apium graveolens* can be source for production of cyclodecane.

Keywords: callus, secondary metabolites, tissue culture, Apium graveolens
Title: miRNA, a new method for hemophilia B treatment

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Abstract: Introduction: Hemophilia B is a bleeding disorder characterized by a deficiency in coagulation factor IX. Carboxylation of glutamate is essential for production of active hFIX is done by γ-carboxylase enzyme which is inhibited by Calumenin. RNAi has now gained popularity in a variety of biological systems as a methodology of choice for knocking down gene expression. Our main goal in the present study is designing artificial miRNAs against human Calumenin and studying the application of artificial miRNAs to silence the inhibition of γ-carboxylase activity by calumenin.

Methods: Calumenin variants Sequences were obtained from NCBI. Using dharmacon, we designed siRNA for common sequences. The sequence of siRNA was replaced in the miR-30 endogenous miRNA pri-precursor backbone. Then this sequence analyzed by mfold program. Artificial miRNAs were synthesized by Shingene company in pUC57. We were attached artificial miRNA at the 3’ end of hFIX cDNA in PCDNA3 vector by molecular techniques.

Results: The result of mfold showed that the designed sequences could form stem–loop structures. Cloning of artificial miRNA at the 3’ end of hFIX cDNA was confirmed by digestion and electrophoresis. After verification, the recombinant plasmids are considered for transient expression of the hFIX in mammalian cell line in parallel with cell transfected with parental hFIX expression plasmids.

Conclusion: Based on the results reported previously, which showed a 5-fold increase in γ-carboxylase activity, following the application of RNAi, an improvement of the hFIX expression efficiency is expected after expression of the artificial miRNA.

Keywords: Hemophilia B, artificial miRNA, calumenin.
Title: The Study of association between C/T polymorphism in intron region of FGFR2 gene and breast cancer

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Abstract:
Introduction: Breast cancer is the most common cancer in women. Genome-wide associated studies have revealed polymorphisms in FGFR2 gene that may be associated with breast cancer. Fibroblast growth factor receptor2 (FGFR2) plays a pivotal role in cell growth, invasiveness, mammary gland development, cancer and angiogenesis. Over expression of FGFR2 gene is associated with breast cancer. Two independent studies have been identified several SNPs in second intron of FGFR2 which are associated with breast cancer in Europeans. These SNPs could be increased the expression of FGFR2 gene. SNP (rs2981582) would upregulate FGFR2 expression in breast cancer tissues by altering RUNX2 or C/EBP binding affinity. We study the association between rs2981582 in FGFR2 and risk of breast cancer in Isfahanian population.

Methods: Genomic DNA was extracted from blood sample of 80 patients with breast cancer and 80 healthy controls. Tetra-primer ARMS-PCR was used for genotype detection of C/T polymorphism in FGFR2 gene. Primers were designed for rs2981582 using oligo software. Intronic region of FGFR2 was amplified by PCR and 2 allele specific amplicons separated by gel electrophoresis.

Results: We have detected genotype of FGFR2 gene C/T polymorphism in breast cancer patients and control cases by gel electrophoresis. The relationship between breast cancer and this SNP is under investigation.

Conclusion: If our results show relationship between the C/T polymorphism and breast cancer then Tetra-primer ARMS-PCR could be used for early diagnosis and treatment purpose of breast cancer.

Keywords: Fibroblast growth factor receptor2, Single nucleotide polymorphism, Breast cancer.
Title:
In Silico computational analysis on the possible role of miR-26a in Th17 differentiation

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Abstract:
Introduction: Th17 cells are defined as a new subset of CD4+ T-helper cells in immune system which differentiate from naive CD4+ T cell and have been demonstrated to play a critical role in pathogenesis of different autoimmune disease such as multiple sclerosis, rheumatoid arthritis and etc. by now several signaling pathways and their downstream positive and negative regulators involved in Th17 differentiation have been discovered. miRNAs are a new group of non-coding RNAs which take part in post-transcriptional regulation of gene expression by attaching to 3'UTR of their target mRNAs and inhibition of their translation. It has been demonstrated that miRNAs function in control of various cellular processes including differentiation, proliferation, apoptosis and etc. recently it has been reported than miR-26a is significantly up regulated in peripheral blood mononuclear cells (PBMCs) and IL-17 producing cells of patients with rheumatoid arthritis compare with healthy subjects. Methods: by using ten different bioinformatic database designed to predict miRNA-mRNA interaction with various algorithms, we analyzed possible inhibitory effects of miR-26a on positive and negative regulators of Th17 differentiation discovered by now. Results: our results show that miR-26a may have an inducing effect on Th17 differentiation by targeting several negative regulators of Th17 differentiation (such as TSC1 and etc). these results are consistent with previous studies which have reported the up regulation of miR-26a during in vitro Th17 differentiation. Conclusion: according to our results miR-26a could have an inducing role in Th17 differentiation. however in vivo and in vitro experiments are needed to confirm our computational analysis as it's an ongoing research effort of our team.

Keywords: miR-26a, Th17 subset, Differentiation, Autoimmune disease
Title: Phage display peptide library as a selective approach for isolating PC3 specific peptides

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Abstract: Phage display is a well-established approach for the identification of bioactive therapeutic peptides through the use of high diversity libraries. These peptides present several clinical advantages, including specificity and selectivity. Thus, phage display can be used to generate 'targeted therapeutics', which is the current clinical paradigm of choice in the field of oncology. Prostate carcinomas belong to the most widespread tumors, and their number is increasing. Cell surface proteins of prostate cancer cells are especially important. Identification of ligands for these proteins will allow use of these ligands as potent diagnostic and therapeutic tools. The aim of this study was to identify peptides that specifically targeted prostate cancer cells were chosen as target for screening through phage peptide library. For this purpose, a phage display 7-peptide library was exploited for biopanning. Following a number of rounds of biopanning, several phages were obtained with the ability for specific binding to target cells. The ELISA technique was exploited to test the specificity of isolated phages towards prostate cancer target cells. Currently, we are analyzing the data obtained by sequencing of isolated peptides via bioinformatic tools. Furthermore, determining the sequence of peptides displayed on the surface of phages is currently under performance. The selected peptides can be used for prostate cancer diagnosis in urine and blood of patients thereby representing potential for finding novel therapeutic approaches for prostate cancer.

Keywords: Phage display, Screening, Targeting prostate cancer
Title:
Fabrication and characterization of bio scaffold based on modified PVA/zeolite nanocomposite having bone regeneration potential by electrospinning.

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Abstract:
Poly(vinyl alcohol) has been used in practical applications because of its excellent chemical resistance, physical properties, excellent biocompatibility and biodegradability. The suitability of PVA as a tissue engineering scaffold has been a debatable topic as many groups claim that the hydrophilic nature of PVA makes its surface subpar for cell attachment but PVA cross-linked by Glutaraldehyde shows a more hydrophobic surface which facilitates protein adhesion followed by cell adhesion.

Due to more mimicking the scaffold structure to ECM, in this study we used a nanoparticle deserving adsorption of hydroxyapatite when faced with simulated body fluid named zeolite A. Zeolite A increases proliferation, differentiation, and transforming growth factor β (TGF β) production in normal, adult human osteoblast-like cells in vitro. In concentrations from 0.1 to 100µg/mL, zeolite A induces a dose-dependent increase in DNA synthesis of normal human osteoblast-like cells. In order to fabricate scaffold made of above described nanocomposite, we used proper solution of PVA then dispersing mentioned amounts of zeolite A to yielding a homogeneous solution by adding optimum amounts of glutaraldehyde as a cross linker during magnetic stirring then adding HCl as a catalyst for cross linking of polyvinyl alcohol chains. This solution was loaded into plastic syringe and electrospin machine started to inject a solution toward metallic collector coated by aluminum foil. Resulted scaffold showed tune characteristics for cell culture.

Obtained scaffold was totally dried in a vacuum for furthermore analysis including wettability, bioactivity, FTIR, XRD and SEM.

Keywords: scaffold, polyvinyl alcohol, zeolite A, electrospinning
Title:
Investigating the Mechanism of Selective Transport through the Nuclear Pore Complex (NPC)

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Abstract:
Introduction: The NPCs are pathways connecting the nucleoplasm and cytoplasm, selectively control the passage of macromolecules through the nucleus. Small molecules diffuse freely across the nucleus, while large molecules are only allowed to pass when bound to transport receptors. The NPC is made up of 30 types of proteins called nucleoporins (Nups). The FG-nups are intrinsically unstructured, that contain phenylalanine-glycine repeating sequences (FG-repeats). The unstructured nature of FG-nups and their collective behavior is important to understand the NPC's selective transport. In this paper a coarse-grained molecular dynamics simulation has been used to study a representative volume of the FG-nup-filled central pore.

Method: In this research, one of FG-nups named nsp1, is divided into 25 segments, each containing 100 amino acids. The 25 segments were tethered onto a planar surface to mimic the anchoring of FG-nups onto the NPC central surface, forming a 5 × 5 array. We setup a coarse-grained molecular dynamics simulation of the individual 100 amino acid segments and also array with ESPResSo package and compare the results to elucidate the mechanism the NPC transport.

Results: Molecular dynamics simulations of these two systems shows that the individual segments form globule-like structure while 5 × 5 array adopted a brush-like structure, adding transport receptor (NTF2), lead to the collapse of brush-like structure.

Conclusions: results show the brush-like structure adopted by an array of nsp1 is able to distinguish NTF2. the best model to describe the mechanism of NPC is the virtual-gate model, which proposes FG-nups form an entropic barrier, a dense array of FG-nups is attached to the surface of the NPC channel. While transport receptors can carry large molecules bound to them through this barrier by binding to FG-repeats, but inert molecules are excluded.

Keywords: NPC, nsp1, molecular dynamics simulation, coarse-grained model, brush-like structure, selective transport.
Title:
Green synthesis of silver nanoparticles by Linum album, Linum flavum, and Stevia rebaudiana

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Abstract:
Development of green nanotechnology is of great interest for the researchers to achieve ecofriendly biosynthesis of nanoparticles. Silver nanoparticles play a significant role in the field of biological and medical sciences due to its attractive physiochemical properties. Biological methods for synthesis of nanoparticles using plant extract have been suggested as possible ecofriendly alternatives to chemical and physical methods. In this study, silver nanoparticles (AgNPs) were prepared by three different plant species, Linum album, Linum flavum, and Stevia rebaudiana. Aqueous extracts of each plant (30 ml) was treated with 90 ml of silver nitrate solution (10 mM) and incubated for a definite time interval. The characteristic surface plasmon resonance of the nanoparticles was monitored at 420 nm by spectrophotometer. The samples were then purified by several rounds of centrifugation at 12,000 rpm for 20 minute. Size and morphology of the nanoparticles was analyzed by scanning electron microscopy (SEM). Comparison of the typical SPR band of nanoparticles showed that AgNps are produced with a faster trend in the presence of Linum flavum extract. Results of this investigation could encourage production of other types of nanoparticles via green synthesis techniques, for various biomedical and biological applications

Keywords: silver nanoparticles, surface plasmon resonance, green synthesis
Title:
An Assessment of Radiation Hazard Index and Absorbed Dose from Natural Radioactivity in the Soil of Lahijan Province, Iran

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Abstract:
Introduction: Naturally occurring radio nuclides such as 40K, 226Ra and 232Th which emit gamma radiation through their decaying process could reach the human in vicinity. The study area was chosen for its variety of surface conditions such as slope, flat land and also forest area, which is used as a reference place.

Material and method: 14 soil samples from surface soils of Lahijan city and 28 soil samples from rice fields were collected during spring of 2008. Soil samples were collected using hand auger, and the sampling positions were determined using a Global Positioning System (GPS). The amount of radioactivity concentration of these radio nuclides is the important factor in assessing whether it is harmful or otherwise. In this study, the surface doses rate measurements were done in-situ using dose rate meter, and the radioactivity concentration levels were done by counting the soil samples using gamma spectrometer with HPGe detector in the laboratory. The amount of uranium, thorium and potassium in soil were determined using neutron activation analysis (NAA) technique.

Results: Absorbed dose rate in air values obtained in this study, with the mean value of 63.76(49.55-75.96) nGy / h in soil samples were obtained. $H_{ex}$ Results in soil samples in the area range from (0.28- 0.43)$Bq kg^{-1}$ with 0.361 $Bq kg^{-1}$ mean.

Conclusions: The results show a reasonably low radiation absorb dose and radiation hazard index, which is a good indication for the farmers to work in the area.

Keywords: Radio Nuclides, HPGe Detector, Soil, Gamma Radiation,40K, 226Ra and 232Th
Title:
Insulin under hyperglycemia and hyperketonemia

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Abstract:
Introduction: One of the consequences of the hyperglycemia associated with diabetes is the non enzymatic glycosylation (glycation) of proteins. Glycation leads on conformational and functional alteration of the proteins that generally bring about fibril formation. However, it is important that raising glucose concentration is accompanied by increasing of the concentrations of keton bodies (KBs) emphasizing on diabetic type 1 condition. We are presenting the effect of glucose or KB or joint presence of them on the structure and function of the insulin.

Material: The secondary structure was assessed using CD and tertiary structure was determined using Intrinsic fluorescent and ANS. Kinetic of fibril formation were monitored with ThT.

Result: A dramatic increase in ANS fluorescence and a decrease in intrinsic fluorescence of the glycated insulin with glucose were observed. However, brief change in ANS fluorescence insulin glycated with KB or KB+Glc was seen. Rate of fibril formation in the presence of KB or KB+GL was resulted to be slower than Glc alone. Secondary structure of glycated insulin was changed in which α to β structural change was observed while KB preserved native conformation.

Discussion: Our results showed that KB retains the native structure of the insulin during glycation and make the protein less favored to form fibril but Insulin glycation in the absence of KB bring about loss of the secondary and tertiary structures to form partially folded intermediate which is the precursor for fibril formation. These intermediates are conformation that substantially associated to form insulin amyloid like fibril.

Keywords: Insulin, Glycation, GLC, Ketonbody
Title:
Role of γG XmnI polymorphism in thalassemia intermedia patients

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Abstract:
Introduction: Beta Thalassemia is the most common inherited bloody disorder, affecting synthesis of the beta globin chain of hemoglobin. The presence of XmnI polymorphic site at the 5´ region of the γG-globin gene affects on the rate of γG-globin synthesis and also on production of HbF. Therefore these events are important in the view of presentation of clinical symptoms in β-Thalassemia patients.

Materials and Methods: In present study 51 beta Thalassemia Intermedia (TI) patients were studied. The XmnI polymorphism in the region of 5´ to γG gene was determined by Tetra Primers ARMS-PCR technique. Finally the samples were analyzed by agarose gel.

Results: So far we have observed bands in expected length at electrophoresis using designed primers in PCR technique, for patient samples. The polymorphism association with TI is under investigation.

Conclusion: XmnI polymorphism was observed in TI patients. According to our present observation (not statistically) XmnI polymorphism has association with reduction of clinical symptoms.

Keywords: Thalassemia, HbF, XmnI polymorphism, Tetra Primers ARMS-PCR
Title: Synthesis, Characterization, Cytotoxicity of Palladium (II) Complex of Oxaliplatin analogue

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Abstract: Oxaliplatin, (trans-R,R-cyclohexane-1,2-diamine) oxalatoplatinum(II), has recently been approved for combination chemotherapy of metastatic colorectal cancer. Oxaliplatin is a platinum containing antineoplastic agent. It is thought to exert its cytotoxic action in a similar manner to alkylating agents by causing inter- and intrastrand cross links in DNA, inhibiting DNA synthesis and inducing apoptotic cell death. In vivo studies showed that Oxaliplatin has anti-tumor activity against colon carcinoma through its cytotoxic effects. In order to setup structure-activity relationships and to explore the possibilities of improving the anticancer activity of oxaliplatin, which was recently approved for combination chemotherapy of metastatic colorectal cancer, new oxaliplatin analogues have been synthesized and studied. In addition, the cytotoxicity of some palladium compounds is thought to result from inhibition of DNA synthesis in cancer cells. In this paper, we are synthesized oxali-palladium with new green method (solvent is just water). This complex has been characterized by spectroscopic methods such as UV-Visible, FT-IR and ¹H-NMR as well as conductivity measurements and CHN analysis. Also, its antitumor activity was also tested in vivo against leukemia K562 cell line.

Keywords: Oxaliplatin analogues, palladium complex, Cytotoxicity, leukemia K562.
Title:
Investigation of the interaction of vitamin D3 with bovine alpha-lactalbumin

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Abstract:
Vitamin D is a kind of lipid-soluble vitamins, which is essential for maintaining the level of calcium in blood (serum). The previous research shows that a high level of vitamin D in the human blood has a positive effect on health and decreases the risk of cancer. Alpha lactalbumin is one of the major protein components of milk, which has an affinity for ligation to a group of hydrophobic ligands like, some fatty acids, retinol and some peptides. Interaction of vitamin D3 with bovine alpha lactalbumin was investigated by using different spectroscopic methods. Quenching of protein intrinsic fluorescence and an alteration of circular dichroism (CD) spectrum showed that the interaction of proteins with vitamin D3 causes conformational changes as well as a small secondary structure change in alpha lactalbumin. UV-Vis, fluorescence and CD spectroscopic data supported the formation of a complex of D3 and alpha lactalbumine. Binding of Vitamin D3 to alpha lactalbumine can be very beneficial for entering this hydrophobic vitamin into the aqueous phase and enrichment of foods.

Keywords: Alpha-lactalbumin, Vitamin D3, fluorescence, Circular dichroism, hydrophobic ligands
Title:
study of allele frequency and heterozygosity of RS747781 in ATP7B gene

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Abstract:
Introduction: The ATP7B gene produces a membrane protein that regulates the transport of copper out of cells. mutation in this gene caused Wilson disease(WD). Wilson disease caused copper accumulates in tissues. molecular diagnosis of the disease using polymorphic markers, which are located in the ATP7B gene region is favorable. therefore, investigation for informative markers in the ATP7B gene region is an important step in linkage analysis and molecular diagnosis of the disease in Iranian population.

Method: In the present study by use of different bioinformatic tools, a single nucleotide polymorphisms (SNP) marker (RS747781) at the 5’ region of in the ATP7B gene were investigated. after that, four primers were designed by using Oligo primer software. Genomic DNA was extracted from the peripheral blood of 150 unrelated individuals and 40 family members of control Iranian people and this region amplified by Tetra ARMS PCR technique.

Result: the results of DNA amplification were analyzed on Agarose gel. after genotyping of the marker, allele frequency and heterozygosity of the marker were obtained.

Conclusion: Due to the importance of ATP7B gene and different mutations which are related to the WD, introduction of informative molecular markers in this region and genotyping them could be useful to determine their phase and heterozygosity and following, molecular diagnosis of the disease in Iranian population.

Keywords: ATP7B gene, RS747781, Wilson disease, ARMS PCR
Title:
Non-enzymatic glycation of insulin reduces its propensity for aggregation

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Abstract:
Insulin hormone is associated with a clinical condition known as insulin injection amyloidosis, characterizing by formation and tissue deposition of amyloid fibrils. The milieu within pancreatic β-cells represents a particularly favorable environment for protein glycation. Recent evidences with the animal models of diabetes demonstrated that during different stages of synthesis and storage, a substantial proportion of proinsulin and insulin become glycated. In this study, insulin has been subjected to the non-enzymatic glycation under both reducing- and non-reducing conditions. The glycated insulin sample and its non-glycated counterpart were used for the structural analysis and the stress-induced aggregation. Different spectroscopic techniques and mobility shift electrophoresis under reducing and non-reducing conditions were applied to assess the structural alteration and oligomerization of insulin molecules as a result of non-enzymatic glycation. While non-glycated control sample of insulin easily get aggregate under chemical stress, its corresponding glycated form resists to different extent against amorphous aggregation, and the degree of resistance was correlated to the level of insulin glycation. Also the results of gel mobility shifts suggest a significant role for disulfide cross-linkings in the oligomerization of both glycated and non-glycated insulin samples. Insulin glycation may induce different structural constrains which resist against stress-induced aggregation of this protein. The proper binding of this hormone to its membrane receptor is highly important in insulin action, insulinclearance and the development of insulin resistance. The glycated insulin molecules may demonstrate an altered ability for the receptor binding which affect different biological functions of this glucose regulating hormone.

Keywords: Insulin, Aggregation, Glycation, Spectroscopic analysis, Gel mobility shift
Title: Time Optimization and Antioxidant Activity of Protein Hydrolysates Prepared from Camel Milk Caseins

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Abstract: Antioxidant activity of protein hydrolysates prepared from camel milk caseins using pepsin and proteinase K (PK) was evaluated. The hydrolysis by pepsin and PK was carried out according to the following conditions: a) Pepsin: at pH 2 and 37°C and E/S=1/100 (w/w), b) PK: at PH 8 and 37°C and E/S=1/100 (w/w). The degree of hydrolysis (DH) was determined by measuring the increase in amino groups during the course of the time, using Ophtaldehyde (OPA) solution. Radical scavenging activity (RSA) of hydrolysates was assessed using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). The evaluation of the effects of hydrolysis time on preparing antioxidant peptides up to the 270th minute showed that the RSA of hydrolysates prepared by pepsin increased significantly up to the 90th minute and then reached a nearly stable level. When PK was used to produce bioactive peptides, hydrolysates with highest RSA appeared in the first few minutes of hydrolysis. Study of the effect of the DH on RSA of camel milk casein peptides prepared by pepsin revealed that the RSA of hydrolysates with medium and higher DH was stronger compared to lower DH values whereas hydrolysates prepared by PK revealed higher RSA with lower DH.

Keywords: camel milk casein, antioxidant activity, degree of hydrolysis, time of hydrolysis
Title: Fluorescence imaging of cancer cells by application of novel fluorescent dendro-nized magnetic nanoparticles

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Abstract: 
Introduction: The combination of medical and nanotechnology associated with production of composite material is clearly on the rise. Excitement over these themes has triggered a tumult of activity in nanomaterial design and development as diagnosis and therapeutic agent to boost the clinical benefits of nanomedicine. Method: In this study, we described the synthesis of poly(amidoamine)(PAMAM) dendrimer coated superparamagnetic iron oxide nanoparticles (SPIONs) and then functionalized them with fluorescein isothiocyanate (FITC) with a particular application for fluorescence imagining. The nanocomposites are characterized by UV-vis spectroscopy, FTIR, XRD, TEM and VSM. Results: The FTIR results confirmed the functionalized surfaces of magnetite nanoparticles with PAMAM dendrimer and FITC terminal groups. Based on the XRD results the reflection peak at 2h = 35.60° corresponds to the spinel phase of Fe3O4 was identified. In addition, TEM results showed that the diameter of SPIONs and final products were 10 nm and 14 nm, respectively. A possible fluorescence imaging of MCF-7 cells using magnetic nanocomposites and based on laser-induced fluorescence is demonstrated. In our case the synthesized fluorescent nanocomposites can successfully distinguish the position of cancer cells with aggregation into them or on cell membranes in confocal microscopy imaging results. Conclusions: These findings are promising for potential application of fluorescent dendro-nized magnetic nanoparticles in nanomedicine with respect to their other unique characteristics such as high amount of magnetization saturation (Ms=52 emu/g) which also nominates them as MRI contrast agent.

Keywords: poly(amidoamine) (PAMAM) dendrimer, superparamagnetic iron oxide nanoparticles (SPIONs), fluorescein isothiocyanate (FITC), Fluorescence imaging, nanomedicine
Title:
pH-Sensitive Galactopyranoside Glycol-hydrogels as Drug Vehicles in Oral Delivery Systems

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Abstract:
Introduction: Local or systemic drug delivery to the colon has been achieved by targeting pharmaceutical drugs to this site. The present paper deals with the development of high loading capacity pH-sensitive glycol-hydrogels, using allyl \(\alpha\)-D-galactopyranoside as a biodegradable and biocompatible carbohydrate. New pH-sensitive glycol-polymers have been developed by free-radical polymerization of methacrylic acid and allyl \(\alpha\)-D-galactopyranoside, using 1, 6-hexandiol diacrylate and 1, 6-hexandiol propoxylatediacrylate as cross-linking agents. The new sugar bearing galactopyranoside based hydrogels were analyzed to test their in vitro hydrolytic behavior as potentially drug delivery systems. Method: Equilibrium swelling studies were carried out in enzyme-free simulated gastric and intestinal fluids (SGF, pH 8, and SIF, pH 1, respectively). A model drug, olsalazine as an azo derivative of 5-aminosalicylic acid was entrapped in these gels and the in vitro release profiles were established separately in both SGF and SIF at 37 °C. Results: The swelling and drug release profiles revealed that the amount of drug release and swelling of the hydrogels depended on the content of methacrylic acid and crosslinker type and content. The drug release behavior of hydrogels was significantly affected by polymer composition. It appears that the degree of drug release from network polymers depends on the degree of swelling. There is a notable difference between drug release behavior of copolymers at acidic and basic mediums. Higher release rates at pH 8 are attributed to carboxylic groups of methyl acrylic acid (MAA). At basic medium, ionization of carboxylic acid groups increases hydrophilicity of polymers which increases the penetration of hydrolyzing agent in polymer network. At low pH ranges, the gels have a lower swelling ratio which is due to internal hydrogen bonds between carboxylic acid groups. Olsalazine release behavior of hydrogels indicates that release velocity decreases with increasing the crosslinking agent content. Conclusions: The swelling and drug release behavior of the hydrogels depended on the content of MAA and cross-linker type and content. For all samples swelling and drug release decreased with increasing crosslinker content. Due to the high different hydrolysis rate in pH 1 and 8, these hydrogels are candidates for colon-specific drug delivery.

Keywords: Glycol-hydrogel, Oral drug delivery, Allyl \(\alpha\)-D-galactopyranoside
Title:
A new luminescence method for Hepatitis B virus detection based on gold nanoparticles

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Abstract:

Introduction: Immunosensor is one of the specific detection methods in antigen diagnosis. It benefits from high affinity of antigen-antibody interaction. The sensitive luminescence immunoassay method based on antibody, luminol and gold nanoparticles with HAuCl₄ as catalyst were selected for the detection of Hepatitis B virus. So, the designed immune-sensors can improve the parameter in amplification of the signal by using gold nanoparticles (GNPs) to exhibit remarkable optical properties.

Method: In sandwich type of immunoassay method, the primary antibody immobilized in polystyrene and using the secondary antibody conjugated to streptavidin, the modified antibodies combined with antigen to form a sandwiched immune reaction that was monitored by luminescence detection. For this purpose, GNPs were modified by biotin and luminol that can attach to streptavidin.

Results: The complex amplified the luminescence signal. The luminescent intensity was proportional to the concentration of the antigen in sample. Catalyst comparison proved that the HAuCl₄ is the best catalyst for this type of detections. After optimizing assay conditions, calibration shows the detection limit of the antigen in this immunosensors was 2 pico gr/ml. Some parameters such as the immunoassay parameters, gold amplification parameters and analytical performance could affect on luminescent signal production. In the amplification parameters, AuNPs produce localized surface plasmons, which influence nearby luminophores and an enhancement in their luminescence intensity.

Conclusions: The detection limit of the method is lower than that produced either by the widely used enzyme-linked immunosorbent assay or by the clinical routine chemiluminescence immunoassay.

Keywords: Amplification of luminescence signal, Gold nanoparticles, immunoassay
Title: Investigation on Thermal Behavior of Environmentally-compatible Biomacromolecules Modified by Suger Moieties

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Abstract: Introduction: According to the most commonly used definition hydrogels are three dimensional networks of hydrophilic polymers able to absorb a large amount of water and biological fluids. Recently, the attention of researchers is mainly focused on hydrogel materials based on natural biodegradable polymers. The thermal behavior of hydrogel is important in relation to its properties for controlling the release rate in order to have a suitable drug dosage form. The aim of presented work was synthesis of ionically crosslinked biocompatible Hydrogels by free-radical polymerization of methacrylic acid (MMA) and alyll α-D-galactopyranoside, using 1, 6-hexandiol diacrylate and 1, 6-hexandiol propoxylate diacrylate as cross-linking agents. The new sugar bearing galactopyranoside based hydrogels were analyzed to test their thermal behavior as potentially influence the hydrolyse behavior of the newly synthesized carriers. Method: Changes in chemical structure of alyll α-D-galactopyranoside hydrogels after their ionic crosslinking were analysed by FTIR spectroscopy. The influence of crosslinking process on thermal stability of alyll α-D-galactopyranoside hydrogels was studied using thermogravimetric and differential scanning calorimetry methods. Glass transition temperature (Tg) was determined using a differential scanning calorimeter (DSC, TGA/SDTA 822) at a heating rate of 10 °C/min under nitrogen atmosphere. Results: increasing MAA content in monomer feed resulted in higher Tg, which is due to increasing internal hydrogen bonds between the polymer chains. Also, Tg values increased with increasing cross-linking density. This is due to decreasing flexibility and mobility of polymer chain. Diffusion rate of small molecules through polymer matrix is often lowered with the increasing Tg for the increasing restriction of chain-segment mobility. By analyzing the thermal behavior of carriers containing a drug model, olsalazine [3, 3'-azobis (6-hydroxy benzoic acid)] (OSZ), in is revealed that Tg can influence the OSZ release behaviors of hydrogels. Conclusions: Changes in chemical structure of newly synthesized hydrogel after their crosslinking were analysed by FTIR spectroscopy. The influence of crosslinking process on thermal stability of hydrogels was studied using differen-tial scanning calorimetry methods. The Tg of composites in this study are higher than 142 °C, which is much higher than the temperature of release study. Therefore, the effect of Tg difference may be less important for drug release study. Keywords: Hydrogel, Crosslinking, Alyl α-D-galactopyranoside, Thermal stability, DSC
Title:  
Effect of drought stress on the PR gene expression of Mentha pulegium L.

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Abstract:  
Introduction: Pulegone reductase (PR) is one of the genes in the biosynthesis pathway of monoterpenes in Mentha genus. The expression level of PR gene was studied under drought stress in Mentha pulegium.

Method: Six weeks after sowing, seedlings were grown under soil moisture corresponding to 100, 75, 50 and 25% field capacity (FC) for the next four weeks. The expression level of PR gene was measured by semi quantitative RT-PCR.

Results: The expression level of PR gene increased under drought stress until 50% FC and then decreased at 25% FC. The highest gene expression of PR was observed at 50% FC.

Conclusion: It seems that drought stress can induce the biosynthesis pathway of monoterpenes compounds in Mentha pulegium.

Keywords: Gene expression, Drought stress, Pulegone reductase, Mentha pulegium
Title:
Destructive effect of fructation on hemoglobin via oxygen transporter molecule (heme)

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Abstract:
Nonenzymatic cascade of reactions between sugars and protein’s amino groups is known as glycation. This modification of proteins causes structural and functional changes which are important in many molecular bases of important diseases such as diabetes. Long live proteins such as hemoglobin (Hb) are susceptible targets for these alternations especially in hyperglycemic conditions. Red blood cells of diabetic patients are an accumulation place for fructose that is so reactive for glycation reaction which is called fructation. To investigate the destructive effect of fructation on oxygen transporter molecule, heme, we used fluorescence technique in an in-vitro incubation of normal and diabetic ratio of Hb and fructose. Two fluorescent heme degradation products by 321 and 460 nm excitation and 430 and 525 nm emission characterization were monitored before and after dialysis. This monitoring indicates the presence of heme degradation products before and after the dialysis. Also the existence of emission spectra with different excitation characterization in the range of 300-400 nm confirm the presence of multiple fluorescent agents in the environment at time more than 20 days. Our results showed not only a time dependence manner for product accumulation, but also the attachment of heme degradation products to the Hb in diabetic patients. It seems that using different drinks and comestibles in an industrial world needs more attentions because in such life, diabetic risk factors and consequential events are more probable.

Keywords: Glycation, Hemoglobin, Fructose, Heme Degradation, Fluorescence
Title:
A Metabolomics study on the alcoholic extraction of turmeric on growth and reproduction of Raji cells using 1HNMR

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Abstract:
Cancer is one of the causes of mortality in humans. Correct and timely diagnosis and elimination of cancer cells is among paramount issue that must be noticed. As medicinal herbs have been reported to have anti-cancer effects, many trials are on to use them either with or without chemotherapy. Turmeric is a medicinal herb which contains the active compound of curcumin which has different anti-cancer, anti-tumor and anti-inflammatory properties and is used in a wide range of diseases. In this study, Raji cells were cultured in RPMI 40 with and without curcumin to determine their IC50. They were extracted by chloroform and two phases of hydrophilic and lipophilic phases were obtained. They were then analyzed by 1HNMR using CPMG Spin echo method and the data from the two phases were analyzed using MaTLAB with PCA and PLS methods. The relevant metabolites were then identified using the Human metabolome database (HDMB) and the important metabolic cycles were identified using Metaboanalyst database. The significant metabolites identified in hydrophilic phase had reduced. The relevant metabolic cycles in the hydrophilic involved nitrogen metabolism, cysteine-methionine, pyrimidine, purine and pyruvate. These cycles have been earlier reported as having increased in cancer cells. This study shows that curcumin has anti-cancer properties and inhibited the growth and reproduction of Raji cells in vitro.

Keywords: turmeric, curcumin, metabonomics, PLS, Raji cells, 1HNMR.
Title:
Isolation of antibacterial fractions from Iranian honeys by HPLC

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Abstract:
Introduction: Honey has been used since ancient times, both as safe food and medicine. The emergence and spread of antibiotic resistance for example in wound infection causative bacteria has provided the groundwork for alternative natural origin treatments such as honey. Iran has special climate and unique native flora and therefore specific honeys, but antimicrobial properties and effective components of Iranian honeys have been paid less attention. The aim of present study was a qualitative analysis of honey bioactive compounds.

Method: In this study, we surveyed honeys for their antimicrobial properties against four infection causative bacteria in wounds (Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Enterococcus faecalis ). A solution of the approved antimicrobial honey was dialysed using 12.5 kDa membrane. The resulted dializate was freeze dried then fractionated by preparative High Performance Liquid Chromatography (HPLC). Finally collected fractions were surveyed in bioassay.

Results: Among 10 repeatable fractions with maximum absorbance in 220 nm, two fractions were resulted with significant antibacterial activities. According to OPA methods for determining of peptide concentration and their absorbance behavior, they represent to be antimicrobial peptides.

Conclusion: We conclude that many Iranian honeys have clinical values, and that further studies into the identification and characterization of their bioactive constituents are warranted.

Keywords: Iranian honey, Antimicrobial activity, Bioactive compounds, Wound infection
Title: Study and Survey of covalent attachment of Bacteriorhodopsin as Nano Protein Memory

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Abstract: Bacteriorhodopsin (BR) is the light harvesting and photoactive proton pump found in the membrane of a salt marsh bacteria. This protein has significant potential for use in optical computing and memory devices due to unique intrinsic photo physical properties and bioelectric. All these features make the BR one of the most promising protein candidates in protein memories. Protein memory is a kind of optical memories with a large storage capacity and high speed processing features. In the present work we have studied the covalent immobilization of BR on the polycarbonate layer of the CD instead of the dye surface with reading and writing ability.

Methods: The polycarbonate surface was modified by nitration and thus the amine functional groups were attached to the layer. Bacteriorhodopsin suspension 3.2 mg/ml and film1% was immobilized on the modified polycarbonate. The BR activity was detected in three steps. The protein was stabilized and a pH change was examined.

Results: The films contain bacteriorhodopsin showed better activity, and maintained the stability. In this method bacteriorhodopsin stabilized by covalent bonding. This is an appropriate method for optimal stability and activity of the protein and also is suitable for preparation of protein CDs. AFM imaging was also performed.

Keywords: Bacteriorhodopsin, Protein memory, polycarbonate
Title:
Structural studies of thermal inactivation and aggregation of lysozyme in the presence of spermine in neutral pH

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Abstract:
Proteins tend to form undesirable and uncontrollable aggregates at high temperature. Protein aggregation is a major problem in both biological and biomedical field involving proteins. In this study, we show that polyamines, such as spermine, effectively prevent thermal inactivation and aggregation of hen egg white lysozyme, a positively charged protein (pI = 11). In the presence of spermine, even in low concentration, after heat treatment at 98 °C for 30 min, no aggregates were observed. In other word, with increasing of spermine, lysozyme aggregation was decreased from 65% to 3%. The residual activity after this heat treatment was about 50%, while it was very low (<5%) in the absence of additives after the same heat treatment. The midpoint temperature of thermal unfolding (Tm) of lysozyme in the presence of spermine, after heat treatment, was increased with increasing of the concentration of this additive. These results imply that spermine is the most prominent additives for preventing the thermal aggregation and inactivation of heat-labile proteins.

Keywords: Spermine, Aggregation, Thermal inactivation, Lysozyme
Title:
Regulation of β-Catenin signaling by the Gq class of heterotrimeric G proteins

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Abstract:
Introduction: β-Catenin, the vertebrate homolog of the Drosophila Armadillo protein, is primarily known as a component of the canonical Wnt signal transduction which determines early embryonic axis in Xenopus laevis and probably many other vertebrates. In addition, activation of β-Catenin plays a crucial role in the initiation of human colorectal cancer. Most colorectal tumor cells harbor inactivating genetic mutations in the tumor suppressor gene, APC, which result in protein stabilization and abnormal activation of the proto-oncogene, β-Catenin. Therefore, regulation of β-Catenin function could be beneficial to patients suffering from colon cancer or many other cancers in which β-Catenin is upregulated.

Methods: Different cellular, molecular, and biochemical assays have been used for this study including cell culture and transfection, immunofluorescence microscopy, western blotting, GSK-3 kinase assay, immunoprecipitation, gene cloning, PCR, RT-PCR, real-time PCR, etc.

Results: The results from my laboratory suggest that activation of the Gaq class of Ga proteins positively regulates β-Catenin. We have shown that expression of the wild type or the dominant active mutant of Gaq (GaqQL) in HEK293T cells or Xenopus oocytes leads to inhibition of GSK-3β and cellular accumulation of β-Catenin. Activation of endogenous Gaq in HEK293T and HT-29 colon cancer cells by either expressing M3-muscarinic acetylcholine receptor or treating these cells with thrombin (or carbachol) leads to an increase in endogenous cytoplasmic β-Catenin protein levels followed by upregulation of several β-Catenin-target genes. We have also shown that the Gaq-mediated cellular accumulation of β-Catenin can be blocked by expression of a minigene encoding a specific Gaq inhibitory peptide.

Conclusion: Our results open a new avenue toward clinical studies of colon cancer and other human cancers which their initiation and progression are dependent on β-Catenin function.

Keywords: Gaq, β-Catenin, human cancer.
Title:
Study of Interactions Between Hemin and DNA Quadruplex Using Molecular Dynamics Simulation

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Abstract:
Introduction: DNAzymes are single stranded DNA molecules that exhibit catalytic activity and are exploited in medicine, biology and material sciences. In recent years, the development of DNAzyme has received increasing attention because of the many advantages of DNAzymes over conventional protein enzymes, such as thermal stability and simpler preparation. One of the most important DNAzymes is the G-quadruplex-hemin complexes with peroxidase activities. Quadruplexes can exhibit many different topologies. It was reported that there is relationship between G-quadruplex structure and the catalytic activity of DNAzymes, which was related to the ability to bind hemin. Here, the interaction between hemin and G-quadruplex DNA was investigated by molecular dynamics simulation.

Method: In this study, we simulated the interaction between a typical G-quadruplex with the sequence of "GGGTGGGTGGGTGGGT" and hemin by Amber molecular dynamic package. We have simulated the molecules in physiological conditions. The molecules were placed in a truncated octahedral TIP3P water box and neutralized by K+ ions. The salt concentration of 100mM KCl was added to the simulation environment.

Results: A stable state forms after running the simulation for a few nanoseconds. The complex is stable when hemin lies on top of the plane. The structure becomes more stable upon interaction of hemin with backbone of the DNA mainly phosphate groups.

Conclusion: Hydrophobic and electrostatic interactions are important in the binding of hemin to the quadruplex DNA.

Keywords: DNAzymes, Hemin, G-quadruplexs, Molecular Dynamic Simulation
Title: Enhancement of chondroitinase ABC I thermal stability through solvent engineering

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Abstract:
Introduction: Chondroitinase ABC I (cABC I) from Proteus vulgaris cleaves glycosaminoglycan chains which are responsible for most of the inhibition of axon regrowth in spinal cord injury. The clinical utilization of this enzyme is mainly limited by its thermal instability. This study has been undertaken to determine the effects of glycerol, sorbitol and trehalose on cABC I activity and thermal stability.

Method: In this study, the gene encoding cABC I cloned into pET-28a vector. Then the recombinant vector was transformed into BL21 E.coli cells. Colonies were screened by PCR, restriction digestion and sequencing. A positive colony was used for protein expression under IPTG induction. Recombinant cABC I was purified using Ni-NTA chromatography. Then cABC I activity and thermal stability were measured in the presence of glycerol, sorbitol and trehalose as cosolvents. Fluorescence quenching and circular dichroism (CD) studies were also performed to investigate the effect of these cosolvents on cABC I structure.

Results: The results indicated that enzyme catalytic activity and intrinsic fluorescence intensity increased in the presence of these cosolvents whereas no considerable conformational changes observed in far-UV CD spectra. Thermal CD experiment revealed an increase in Tm of cABC I in the presence of these cosolvents which was significant for trehalose.

Conclusions: Our results support the idea that cABC I has stabilized in the presence of glycerol, sorbitol and trehalose.

Keywords: Chondroitinase ABC I, Thermal stability, Solvent engineering, Cosolvents
Title:
Investigation on the effect of accessible surface hydrophobicity and hydrophilicity on the enzyme stability

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Abstract:

Introduction: Biocatalysts are increasingly employed in chemical processing, but they are not usually tolerant to the presence of organic solvents, extremes of pH and high temperatures. Consequently, the stability of enzymes remains a critical issue in biotechnology. Chemical modification provides a rapid and inexpensive method to stabilize enzymes. Horseradish peroxidase has achieved a prominent position in the pharmaceutical, chemical, and biotechnological industries. In this study, chemical modification of HRP has been carried out using two modifiers introducing extra hydrophobic ad hydrophilic groups on the surface of the protein.

Method: The catalytic activity of HRP was assayed using a color-generating substrates and the increase in the absorbance was recorded by the spectrophotometer. The secondary structure, the tertiary structure and the structure around catalytic group of the protein investigated with circular dichroism, intrinsic and extrinsic fluorescence techniques.

Results: Thermal and chemical stability and tolerance of the enzyme in the organic solvent increases due to the surface hydrophilization , but surface hydrophobization only stabilizes the enzyme against chemical denaturation. In addition, the experiments indicate none of the modifiers increase stability of HRP on the oxidative stress and high pH. Structural investigation implies both anhydrides cause to a slight decrease in compactness of the structure upon covalent binding to accessible lysine residues of HRP.

Conclusion: The success of a stabilization strategy depends on the unfolding condition. On the whole, the surface hydrophylisation improves the enzyme stability more effective than the surface hydrophobization on the different denaturing conditions.

Keywords: Horseradish peroxidase, Stability, Chemical modification, Spectroscopy
Title:
Developing consensus QSAR models of Naphthyridine analogues as anti HIV-1 integrase activity

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Abstract:
Introduction: HIV integrase is a good candidate for developing new inhibitors against AIDS because it hasn't any human homologue. QSAR is very useful method for designing drugs according to the rational drug design. The mathematical models which reflect the structural information of drugs by series of descriptors could be constructed in the QSAR modeling. Method: Dragon5.4 and ACD Labs software used for calculation the descriptors of 67 derivatives of 1,3,4-oxadiazole naphthyridine as HIV-1 integrase inhibitors. The suitable descriptors analyzed and selected by the help of SPSS and MATLAB software according to stepwise and GAPLS algorithms. Based on MLR procedure, two models constructed then improved to Stepwise-MLR (consensus) and GAPLS-MLR (consensus) models by using some of the already developed model descriptors. Both of the models were validated via different statistical approaches. Results: The selected descriptors are significant and they have high correlation with IC_{50} as well as low correlation between each others. Y randomization results indicated that there is not chance correlation between selected descriptors with experimental IC_{50}. Both of the consensus models are high efficiency in prediction of the biological activity for test set compounds. Conclusions: The descriptors of models expressed the presence of amide, alcohol and acid groups that essential for the activities of compounds. The robust of consensus models is depend on the different training set in our study with previous one. Our finding could be help to design new and effective drugs for AIDS therapy.

Keywords: QSAR - consensus model - HIV integrase - naphthyridine
Title:
The effect of vanadyl and gallium diacetylcurcumin complexes on horseradish peroxidase

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Abstract:
Introduction: Curcumin, a natural polyphenolic compound extracted from the rhizome of the herb Curcuma longa has been reported to have a wide spectrum of biological and pharmacological activities including anti-inflammatory, antioxidant, antiproliferative, antiangiogenic, anti-microbial and anticancer activities. The investigations have indicated that the most important reason of the bioactivities of curcumin is related to its strong antioxidant property. Some of antioxidant effect of curcumin is attributed to its interaction with enzymes. Here, to understand the antioxidant properties of vanadyl and gallium diacetylcurcumin (VO(DAC)₂ and Ga(DAC)₂), their effects on horseradish peroxidase (HRP) were investigated. Peroxidase enzymes eliminate toxic hydrogen peroxide from the cell.

Method: The catalytic activity of HRP was assayed using phenol, 4-aminoantipyrine (4-AAP) and H₂O₂ as color-generating substrates and the increase in the absorbance at 510 nm resulting from decomposition of hydrogen peroxide was recorded by the spectrophotometer. To obtain kinetic parameters, the rate of enzymatic reaction was measured in the different concentration of hydrogen peroxide and phenol. In addition, IC₅₀ for hydrogen peroxide as an oxidizing agent was obtained by measuring the activity at different concentration of H₂O₂.

Results: The results indicate that both complexes decrease Kₘ and increase catalytic activity of the enzyme. Furthermore, the complexes increase IC₅₀ for hydrogen peroxide to some extent. For example, IC₅₀ is 3.1 and 4 in the absence and presence of 3µM of VO(DAC)₂.

Conclusions: Ga(DAC)₃ and VO(DAC)₂ reduce oxidative damage caused by a hydrogen peroxide and improve catalytic efficiency of the enzyme.

Keywords: Vanadyl diacetylcurcumin, Gallium diacetylcurcumin, Catalytic efficiency, Hydrogen peroxide
Title: Synthesis, Characterization and Biodegradation of Poly(butylene succinate) scaffolds

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Abstract: With the advances in technology and the increase in the global population, plastic materials have found wide applications in every aspect of life and industries. However, most conventional plastics are non biodegradable, and their increasing accumulation in the environment has been a threat to the planet. There is a world-wide research effort to develop biodegradable polymers as a waste management option for polymers in the environment. Among synthetic polymers, aliphatic polyesters have attracted considerable attention as they combine the features of biodegradability, biocompatibility and physical or chemical properties comparable with many traditional and non-biodegradable polymers. Aliphatic polyesters have found applications in medicine and pharmaceutical technology, for example, in drug delivery systems, surgical sutures, implants, and tissue engineering scaffolds. These scaffolds are made of polymers, ceramics or composites. One of the most promising biodegradable aliphatic polyesters is poly (butylene succinate), PBS, which is produced by the polycondensation of 1,4-butadiol with succinic acid. PBS is commercially available as a thermoplastic polyester with many interesting properties, including biodegradability, melt processability and thermal and chemical resistance. This polymer has been recently proposed for bone tissue engineering. In this research we will try to synthesis poly(butylene succinate) by melt polycondensation method. After that we will prepare a porous scaffold through “solvent casting and particulate leaching” method. Finally the biodegradability of the PBS scaffolds will be investigated.

Keywords: 1 Tissue Engineering 2 Scaffold 3 Poly(butylene succinate) 4 Biodegradable Polymers
Title:
The role of two Gaq agonists (Thrombin and Carbachol) on cellular β-Catenin protein levels and β-Catenin-target gene expression in HT-29 colon cancer cells

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Abstract:
Introduction: Disregulation of the Wnt/β-Catenin signal transduction occurs in colorectal cancer and some other human malignancies. In these cancers, genetic and epigenetic changes in the components of this signaling pathway often leads to abnormal activation of the proto-oncogene, β-Catenin. β-Catenin is a multi-functional protein that in the cell membrane maintains epithelial tissue integrity and in the nucleus, functions as a transcription modulator to regulate transcription of many genes involved in carcinogenesis. The previous results from our laboratory indicate a positive interaction between the Gaq class of Gα proteins and β-Catenin expression and function.

Methods: Here we have extended those studies by treating HT-29 colon cancer cells with two Gaq signaling agonists, thrombin and carbachol. Briefly, HT-29 cells were cultured, treated with the agonists, the cell extracts were isolated, and then cellular β-Catenin protein levels, its intracellular localization, and the target gene expression were measured by western blotting, immunofluorescence microscopy, and reverse-transcriptase PCR respectively.

Results: Exposure of HT-29 cells to thrombin and carbachol led to a dramatic increase in the cytoplasmic levels of the β-Catenin protein (about 10-fold and 8-fold respectively). Thrombin or carbachol treatment of HT-29 cells also enhanced expression of several β-Catenin-target genes including c-myc, cyclin D1, cox2, and the reporter luciferase gene placed downstream of the β-Catenin/Tcf binding elements, although this increase was not more than two-fold.

Conclusions: Collectively our results further support the positive role of Gaq signaling in the regulation of the canonical Wnt/β-Catenin signaling pathway.

Keywords: Colon cancer, Gaq, Wnt signaling, β-Catenin
Title:
Study of the effect of Allicin (an active component of garlic) Sub-MIC Concentrations on Biofilm Formation by Proteus mirabilis

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Abstract:
Background & Objective: P. mirabilis is one of the common agents involved in urinary tract infections, especially in patients undergoing urinary catheterization. P. mirabilis is capable of living as biofilm and/or planktonic forms. Biofilm plays a significant role in P. mirabilis infections. Cells in the biofilm show higher degree of resistance to antimicrobial therapy and host immune responses compared with planktonic cells. So inhibition of biofilm formation could help the body’s immune system to combat the bacteria and improve the clinical outcomes for antimicrobial therapy. This study was aimed to investigate the inhibitory effect of allicin on biofilm formation by P. mirabilis. Material&Methods: This study was carried out using a P. mirabilis ATCC12453. Allicin was purified using semi preparative HPLC procedure. MIC of allicin was determined by microdilution method using serial dilutions of aqueous allicin solution (4-512 µg/ml) in Mueller-Hinton broth. Biofilm inhibition was assayed using Microtiter plate method in the presence of sub-MIC concentrations (4µg-32µg) of allicin. The plates were incubated for 18 hours at 37 ºC. Bacterial biofilms were stained with 0.2% safranin. Dye was solubilized using alcohol-Aceton as solvent and the optical density (OD) was measured at 492 nm wavelength. The extent of biofilm formation was determined (OD of sample well/OD of control well*100). Each assay was performed in triplicate and repeated two times. Results: The allicin MIC was 64 µg/ml for P. mirabilis ATCC12453. The results indicated that allicin at concentrations of 16 and 32µg/ml significantly diminished biofilm formation (P < 0.01). This concentration did not have significant influence on bacterial growth rate. Conclusion: The results showed that allicin can inhibit the biofilm formation by P. mirabilis.

Keywords: Allicin, Biofilm inhibition, P. mirabilis.
Title:
RT PCR of Sox2 due to cancer stem cell investigation

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Abstract:
RT PCR of Sox2 due to cancer stem cell investigation
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Introduction
Cancer stem cell (CSC) was first suggested about 150 years ago by Rudolf Virchow. According to this hypothesis, there are a few stem cells in cancer tissue responsible for initiating cancers and relapsing disease after therapeutic procedures. So these kinds of cells should be proven & detected by their specific markers first. Then, we can design particular therapies that efficiently target these cells to prevent relapse and metastasis. In the latter few years, this theory is completely in doubt in solid tumors. So, as the first step, we should use significant markers to testify this. One of these noticeable markers is Sox2. This transcription factor is highly expressed in stem cells, so up regulation of this in cancer tissue in comparison to normal tissue can be a reliable result to accept CSC theory. In this study, RT PCR is performed for confirming this theory.

Material and method
In the first step, we perform bioinformatics to design specific Sox2 primers. Then, the totals RNA were extracted from 10 breast cancer tissues and first strand of total cDNA synthesized by random hexamer. It is followed by amplification of Sox2 cDNA by specific primers. At last, our PCR products were observed on agarose gel & compared with GAPDH housekeeping gene. Result products were loaded on the agarose gel and sharp bands were seen in 50°C to 60°C.

Conclusion
The certain and final results are under investigation but no significant overexpression have been detected up to now.

Keywords: RT PCR, Sox2, Cancer stem cell
Title:
Study of the association between (C/T) polymorphism in intron 2 of BCL11A gene and HbF level in beta thalassemia intermedia disease

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Abstract:
Introduction: Thalassemias are the most common type of hereditary hemoglobinopathies in the world, that are very heterogeneous at the molecular level. Beta thalassemia is caused by point mutations or rarely deletions in beta globin gene cluster, that leads to decreased synthesis (β+) or absence (β0) of β-globin chains. Increased levels of fetal hemoglobin (HbF) can ameliorate the clinical severity of beta thalassemia intermedia disease. Some polymorphisms in intron 2 of BCL11A gene, a regulator of fetal to adult hemoglobin switching, affect the production and formation of fetal hemoglobin and prevent from clinical symptoms of disease. In the present study, the association between increased levels of HbF and the amelioration of beta thalassemia intermedia disease has been investigated.

Method: Common polymorphism rs11886868 of BCL11A gene was genotyped by Tetra-Primer ARMS PCR method in 50 beta thalassemia intermedia patients. Then PCR products were analysed by agarose gel electrophoresis.

Results: Expected bands were observed and genotyped in samples. Initial observations showed that this polymorphism associated with decreased severity of disease, and it's relationship with HbF expression is under investigation.

Conclusions: Detection of the factors that induce more expression of HbF, is important in beta thalassemia intermedia disease. So if there is a statistical correlation, with initial genotyping of patients, we can informed professionals in prescribing and blood transfusion.

Keywords: Beta thalassemia intermedia, BCL11A, Polymorphism, HbF
Title: Enzyme-like behavior of graphene/silver nanocomposite and its application as electrochemical glucose sensor

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Abstract: The silver nanoparticles coated by graphene oxide (Ag/GO) was synthesized and applied as an enzyme-like nanocomposite for development of electrochemical glucose sensor. The green synthesis of Ag/GO was carried out by one-pot strategy towards rapid preparation of nanocomposite. Silver nanoparticles were adopted by heating the mixtures of GO and aqueous solution of AgNO₃ at an alkaline pH under the constant stirring. The reaction was completed within a short period of 10 min without extra reducing agent. Synthesized Ag/GO nanocomposites have been characterized by UV–visible spectroscopy, powder X-ray diffraction analysis, scanning electrochemical microscopy and cyclic voltammetry. Reduced graphene deposited with silver nanoparticles was applied for amperometric sensing of glucose in alkaline solution. The electrocatalytic oxidation of glucose at modified electrode has been investigated in details. A probable mechanism has been proposed for oxidation of glucose on Ag/GO using cyclic voltammetry measurements. Current-time dynamic response at +0.60 V step potential vs. Hg/Hg₂Cl₂ in NaOH (0.1 M) were recorded and linear response was obtained ($R^2=0.991$) as a function of glucose concentration from 0.2 to 18 mM, with a detection limit of 0.060 mM (signal/noise ratio of 3) and sensitivity of 32 $\mu$A mM⁻¹ cm⁻². The signal corresponding to glucose was not seen to be interfered by ascorbic acid, uric acid and chloride ions present in the solution.

Keywords: Graphene oxide, Electrochemical sensor, Non-enzymatic method, Glucose sensor
Title:
Comparison of catalase activity in leaves and roots with stresses salinity, calcium and gibberellin in Coriandrum sativum L.

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Abstract:
Salinity cause to changes in amount of regular metabolic material and enzymes such as catalase and peroxidase, these changes have a role in giving a tolerance to plants against environmental stresses. Coriander (Coriander sativum L.) is one of the medical and industrial plants that have been investigated less than the other plants in case of salt stress. The aim of this investigation is Comparison of catalase activity in airic organs and roots with salinity, calcium and gibberellin in Coriandrum sativum L. Experiments were conducted based on randomized completely design with 5 treatments and 3 replications. Analysis of data was performed by the statistical program (SPSS) and Duncan test. Treatments were include of NaCl at 4 levels (0, 25, 50, 75 mM/Lit) NaCl at levels (25, 50, 75 mM/Lit), with Calcium (5 mM/Lit), NaCl at levels (25, 50, 75 mM/Lit) with Gibberellin (10 ppm) and NaCl at levels (25, 50, 75 mM/Lit) with Calcium (5 mM/Lit) and Gibberellin (10 ppm). Result show that catalase activity in roots increase significantly, also in addition of calcium (5 mM/Lit) and gibberellin under NaCl stress in roots improve plant salt tolerance.

Keywords: calcium, gibberellin, salinity, Coriandrum sativum L. : calcium, gibberellin, salinity, Coriandrum sativum L.
Title:
The effect of metal curcumin complex on hydrogen peroxide stability and catalytic efficiency of the plant peroxidase

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Abstract:
Introduction: The use of plants as medicines have been important throughout human history. Curcumin as a natural compound possesses diverse pharmacological effects, but the mechanism underlying these diverse effects of curcumin is not fully understood. The therapeutic properties of curcumin have also been considered to be associated with its antioxidant property. It has been previously reported that the metal curcumin complex has a significantly higher antioxidant capacity than natural curcumin. In this study, the effect of gallium curcumin (Ga(cur)$_2$) on the enzymatic activity and hydrogen peroxide stability of the peroxidase as an enzyme with antioxidant activity was evaluated.

Method: The catalytic activity of horseradish peroxidase (HRP) was assayed using a color-generating substrates and the increase in the absorbance was recorded by the spectrophotometer. The catalytic parameters were determined using Michaelis–Menten plot.

Result: A remarkable increase was shown in $k_{cat}$ of horseradish peroxidase in the presence of 3µM of Ga(cur)$_2$. Furthermore, $K_m$ for both substrates of HRP including hydrogen peroxide and phenol decreased due to binding of the enzyme to Ga(cur)$_2$. The investigation of the effect of the complex on hydrogen peroxide stability of HRP implied that the remaining activity of the enzyme in the oxidizing concentration of H$_2$O$_2$ was 50%, but in the same oxidizing condition, the enzyme remained fully active upon interaction with Ga(cur)$_2$.

Conclusion: Gallium curcumin is an effective compound for improving catalytic efficiency and stability of the peroxidae enzyme in the oxidative condition.

Keywords: Gallium curcumin, Catalytic parameters, Stability, Oxidative condition, Peroxidase
Title: Comparative cytotoxic studies of new synthesized Pd and Ni complexes against K562 cell line

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Abstract: Application of metal-organic drugs can be useful in cancer treatment because of positive charges of metal centers which can bind with negative charged biomolecules. So, proteins and nucleic acids are the best targets for them. Despite effective clinical effects observed in platinum compound especially cisplatin, there are some severe side effects which suggest to think about some other metals such as palladium, ruthenium, copper, nickel. Here, we investigated two new designed anti-cancer complex based on [Ni(FIP)2](ClO4)2 and [Pd(Phen)(Pro-gly)]NO3 (where FIP is (2-foran-2-yl) 1H-imidazo-[ 4,5–f] (1,10-phananthroline), Pro-gly is propyl-glycine and Phen is 1,10-phenanthroline.), respectively. To analyze the effect of cell death induction of these complexes the model cancer cell line of chronic myeloid leukemia, K562 was as a target. The cells were cultured in RPMI medium supplemented with 15% FBS and 1% Penicillin-streptomycin, and after some passages, different concentrations of complexes (0-120 mM) for incubation time of 24 h. The cytotoxicity and anti-proliferative properties of the Pd and Ni complexes were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The 50% cytotoxic concentrations (Cc50) of the Pd(II) and Ni (II) complexes were determined 95 and 120 µM, respectively. Results show that the Pd(II) and Ni(II) complexes produced a dose and time – response suppression on growing of K562 leukemia cell lines. Above results suggest that the anticancer metal complex of Pd(II) represent more anti-proliferative activity relative to Ni(II) complex.

Keywords: Pd(II) complex, Ni(II) complex, cytotoxicity, cancer
Title: Study of Degradation a new Poly (ether-urea-urethane) by Bacillus bacteria Isolated from Soil

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Abstract: Biodegradable materials are used in packaging, agriculture, medicine and other areas. In recent years there has been an increase in interest in biodegradable polymers because environmental pollution by synthetic polymers has assumed dangerous proportions, attempts have been made to solve these problems by including biodegradability into polymers in everyday use through slight modifications of their structures.

Conventional polyurethanes (PUs) are among biomaterials not intended to degrade but are susceptible to hydrolytic, oxidative and enzymatic degradation in vivo. Biodegradable PUs are typically prepared from polyester polyols, aliphatic diisocyanates and chain extenders. In this work we have developed a degradable monomer based on α-amino acid to accelerate hard segment degradation. Thus a new class of degradable poly(ether–urethane–urea)s (PEUUs) was synthesized via direct reaction of 4,4'-methylene-bis(4-phenylisocyanate) (MDI), L-leucine anhydride (LA) and polyethylene glycol with molecular weight of 1,000 (PEG-1000) as polyether soft segment.

The present study deals with the isolation of bacteria from soil with the ability to degrade polyurethane (PU). A pure bacteria isolate was analyzed for its ability to utilize PU as a sole carbon source in culture for 30 days. Incubation of PU with Bacillus resulted in reduction in weight of PU. The biological degradability of the polymer was investigated by FTIR, SEM, TGA, DSC and XRD techniques before and after exposure to bacteria “Bacillus”.

Keywords: Poly(ether-urea-urethane), Bacillus, Biodegradation
Title:
Bacillus subtilis genome-wide screening to search sortase and its potential substrates

Authors:
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Abstract:

Introduction: Gram positive bacteria utilize their cell wall for anchoring and display of surface proteins. One important mechanism of protein anchoring utilizes sortase, a transpeptidase which catalyse the covalent anchoring of proteins on the cell wall. Sortase in *S. aureus* recognize an amino acid sequence designated sorting motif, present close to the C-terminal end of the substrate proteins, cleave between T and G residues in this motif and catalyse anchoring of the polypeptide chain to the peptide crossbridge linking the peptidoglycan strands in a transpeptidation reaction. Genome project revealed that *B. subtilis* contain two putative sortases (YhcS and YwpE) but the sorting sequences recognized by them and the substrates has not be known. In this study we were interested in identifying these two types of sortase and their substrates.

Methods: Bioinformatics tools like protein sequence alignment was used to find sortase and its potential substrates in *B. subtilis* genome.

Results: Two different putative sortase genes present in the *B. subtilis* genome, Yhcs and YwpE. Bioinformatics analysis showed that they contain representative domains of sortase family. Also *B. subtilis* has two putative sortase substrates: YhcR and YfkN. Both substrates have a signal sequence at the N-terminal and a cell wall anchoring motif at the C-terminal containing 3 parts (sortase cleavage signal; LPDTS/A, Transmembrane Hydrophobic domain; Charged tail).

Conclusion: YwpE is a 120 aa and YhcS is a 198 aa protein with homology to sortase family and have putative sortase activity. YhcS is more likely to be the more sortase (if any) of *B. subtilis* by structure and map.

Keywords: sortase, substrate, transpeptidation
Title:
Effect of genetic variation on the thermal stability of human serum albumin, a molecular dynamic simulation study

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Abstract:
Mutations of surface residues as well as of amino acids in the interior of proteins can affect their stability. Even single residue substitutions can influence stability as has been observed for, e.g., interacellulary fatty acid-binding proteins, lysozyme, apoflavodoxin and crystallins. In the present work, thermal denaturation of 2 genetic variants of human serum albumin (HSA), 573 Lys→Glu and 82 Glu→Lys have been studied by molecular dynamics simulation. Structure of HSA has been taken from protein data bank (RCSB:1AO6 pdb id code). The thermal stability of these alloalbumins, as compared with that of wild-type albumin, was monitored by analyzing the CD results at 222 nm in the temperature range of 285 to 360 K by Gromacs software. Stability was quantitated in terms of midpoint of the denaturation curve ($T_m$) and vant Hoff enthalpy. In addition, the changes in $T_m$ were related to changes in helical content as is confirmed with structural results and is in good agreement with previous experimental results. The present results can be of both protein chemical relevance and of clinical interest, because they could be useful when designing stable, recombinant HSAs for clinical applications.

Keywords: Molecular dynamic simulation, Mutation, Stability, Denaturation, HSA.
Title:
Polymeric Nano Curcumin, reexpresses silenced tumor suppressor long non coding RNA gene MEG3 via promoter hypomethylation

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Abstract:
Cancer is one of the most substantial causes of mortality worldwide. This renders its prevention and treatment an overwhelming significance. Aberrant epigenetic alterations such as DNA methylation play a significant role in cancer development. Since epigenetic alterations are considered to be more easily reversible compared to genetic changes, epigenetic therapy is potentially very useful in reversing some of these defects. MEG3 is a tumor suppressor long non-coding RNA being expressed in the majority of normal tissues. Yet, methylation of MEG3 promoter region elicits the decrease in its expression in a variety of malignancies. Bioactive nutrients including curcumin offer great potential in altering DNA methylation status with the intention to prevention and treatment of cancer. DNA methylation is catalyzed via DNMT1, DNMT3A and 3B. The fundamental role of altered epigenetic modification patterns in tumorigenesis establishes epigenetic regulatory enzymes as important targets for cancer therapy. Herein, we aimed to investigate epigenetic effects of nanocurcumin on key factors of cancer. The appropriate treatment dose for cells was specified through MTT assay. Following exposure of cells to nanocurcumin, MEG3 expression was examined by q-PCR which exhibited the augmentation of MEG3 expression (p<0.05). Bioinformatic analysis was conducted to designate the promoter regions crucial to epigenetic alteration. DNA methylation assay was studied via bisulfite sequencing technique. Following treatment with nanocurcumin, expression of DNMT1, DNMT3A and 3B, was examined by q-PCR which exhibited downregulation of DNMTs expression (p<0.05). Thus, we conclude that nanocurcumin can induce DNA hypomethylation and re-expression of silenced tumor suppressor genes in cancer cells.

Keywords: Cancer, nanocurcumin, epigenetic, long non coding RNA MEG3, DNMT
Title: Effects of osmolytes on the Protein stability: Molecular dynamics simulation

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Abstract: Introduction: The presences of osmolytes in aqueous solution have significant effects on providing essential interactions for the unique three-dimensional conformation of native proteins. Osmolytes such as trehalose or sucrose have been known to protect proteins against loss of activity and prevent the partial or even total degradation of biomolecules. Despite various experimental and theoretical works, the detailed molecular mechanism at the origin of polyols protective ability still not well understood. MD simulations can provide valuable information about the different stages of peptide–solvent interactions. Methods: All simulations were performed using the gromacs software package, version 4.5.4. The Gromos96 53a6 parameter set was used as force field. The starting conformation of the peptide was obtained from the protein databank (PDB) structure 1LCI. The peptide was solvated with SPC water, a mixture of sucrose, a mixture of trehalose. Results our results showed the total number of hydrogen bonds between the protein and solvents over the course of the MD simulations is not significant Nevertheless there is main difference in water and osmolyte direct interaction with protein. The water molecules mainly interact with protein side chains while osmolytes are more involved with protein backbone. Also, we have identified variations in the secondary structure of the protein during the simulations. In pure water some part of alpha helical conformation of protein is deteriorates with time whereas in sucrose solution protein secondary structure is maintained throughout the 100 ns duration of simulations .Conclusions: In conclusion, according to the results presented in this study it has been suggested that osmolyte with effect on special part of protein and make favorable environment, can prevent protein thermal unfolding.

Keywords: Osmolytes, Protein stability, Protein –co solvents interactions, Molecular Dynamics Simulation
Title:
Investigating the Combined Effects of Aloe Vera Gel and Silver Nanoparticles in Healing Skin Wounds in Rats

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Abstract:
Increasing the rate of wound healing has always been of great interest to medical profession. Aloe vera gel has long been known to have therapeutic effects in skin burns and wound healing. Nanosilver particles are also known to have considerable antibiotic effects. Hence, it was decided to investigate the effects of a mixture of pure extracted aloe gel with nanosilver to see if the mixture would be effective in improving the healing rate of modeled wounds in animal. A known medicinal type of aloe plant was selected from an established greenhouse. After extraction and purification of the Aloe vera gel, a lotion was made by the gel including different concentrations of nanosilver (20 nm). The characteristic of nanoparticles and the lotion stability on the most common causes of infection -Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa- were assessed. Then the silver, gel and the mixed lotion were separately applied to different random groups of seven rats bearing similar dorsally located wounds. The wounds were made in the epithelium and the treatment was carried out for 2 weeks. The size of wound closure was measured by an image software analysis. After closure, animals were sacrificed to obtain tissue sample for light microscopy. All the test groups were compared statistically with the controls to assess the results. Aloe vera group was treated 2 days earlier than the control group. Silver group was treated later than other groups. There is no a significant difference (p<0.05) in wound healing between control and mixture groups, but there are significant differences between silver and Aloe vera groups on days of 6, 8 and10 as well as between control and Aloe vera groups on days 8, 10 and 12. The Aloe vera gel increases the rate of wound healing whereas silver nanoparticles decrease it, but the combined gel has an average effect of both them.

Keywords: Aloe vera gel, Silver nanoparticle, Healing
Title:
Kinetics studies on the bovine liver catalase in the presence of guanidine hydrochloride

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Abstract:
Introduction: Catalase (EC 1.11.1.6) exists as tetramer with four identical subunits that catalyses the decomposition of hydrogen peroxide to water and oxygen. It is an acidic protein, having a higher ratio of acidic to basic amino acid residues. Most of charged residues are found on the outside of the molecule hence, neutralization of these charges by guanidine hydrochloride (GdmCl) should influence the structure and conformation of the enzyme. Therefore activity of Catalase decreased sharply with the increase of concentration of GdmCl. We studied the effect of increasing concentrations of (GdmCl) on the catalytic rate of catalase bovine liver (BLC).

Method: In this study the activity of (BLC) followed by the decreased optical density (OD) of hydrogen peroxide photometrically at 240 nm. Effect of GdmCl as a cationic denaturant on catalase was investigated by UV-Vis spectrophotometry at pH 7 at 25°C using sodium phosphate buffer. Measurements were carried out using 2.75 ×10^-6 gr/ml concentration of catalase and 0.4, 0.6 and 0.8 M of GdmCl concentration.

Results: The result of the present study indicate that the catalytic activity of catalase reduced in the presence of GdmCl. hence, Vmax value will be reduced by increasing of GdmCl concentration.

Conclusions: This study represent useful information for more understanding of the relationship between structural changes and catalytic rate of BLC in the presence of GdmCl. Effect of other cation can be a subject for the next researches.

Keywords: Catalase, guanidine hydrochloride, Spectrophotometry, Conformation
Title:
The computational study of anti-HIV activity of 2- and 3-adamantyl-substituted thiazolidin-4-ones

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Abstract:
In the present study, we have performed QSAR studies. The QSAR studies have been carried out using topological parameters along with thermodynamic and structural descriptors. We have performed QSAR studies for anti-HIV activity of 2- and 3-adamantyl-substituted thiazolidin-4-ones and partial atomic charge and nuclear quadrupole coupling constants (NQCC) of N nucleus on considered molecules. For this purpose the electronic structures and $^{14}$N nuclear quadrupole resonance parameters of 2- and 3-adamantyl-substituted thiazolidin-4-ones have been computed using the density functional theory. The partial atomic charges (Mulliken charges and natural bond orbital (NBO) charges) and nuclear quadrupole coupling constants (NQCC) of $^{14}$N nucleus of the considered molecules have been reported. A reasonable good correlation between NBO atomic partial charges and NQCC of $^{14}$N nucleus and anti-HIV activities has been also suggested.

Keywords: QSAR, NQCC, NBO, Mulliken Charge, DFT
Title: Role of HBS1L-MYB intergenic region polymorphism in thalassemia intermedia patients

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Abstract:

Introduction: The β-globin disorders are among the most common mendelian disorders in humans. Fetal haemoglobin (HbF) level modifies the clinical severity of HBB disorders. HBS1L-MYB intergenic polymorphism (HMIP) on chromosome 6q23 is associated with elevated fetal haemoglobin levels. Therefore this region is important in the view of presentation of clinical symptoms in β-Thalassemia patients.

Material and Methods: In recent study 51 beta Thalassemia Intermediate (TI) patients were evaluated. This particular single nucleotide polymorphism (rs 9399137) that is located on the upstream of MYB gene affects the rate of MYB expression and production of HbF. This SNP was determined by Tetra Primer ARMS-PCR technique and at the end samples were analyzed by agarose gel.

Results: To this point in time we have observed bands in the expected length at electrophoresis using designed primers in PCR technique for patient samples. The polymorphism association with TI is currently under investigation.

Conclusion: The related HBS1L-MYB polymorphism was observed in TI patients. According to our present observation (not statistically) HBS1L-MYB polymorphism has association with reduction of clinical symptoms.

Keywords: Thalassemia, HBS1L-MYB intergenic region, fetal haemoglobin, Tetra Primer ARMS-PCR
Title: Small interfering RNA as targeted therapy of lung cancerous cells

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Abstract: 

Introduction: Telomerase enzyme is expressed in most of malignant cells including lung cancers and do proliferation activity with telomerase reverse transcriptase (hTERT) catalytic subunit. Medical application of siRNA (small interfering RNA) is necessary to efficient, biocompatible, delivery systems to overcome barriers in target sites. A potential siRNA carrier for gene delivery was assessed by encapsulating into biodegradable, biocompatible polyester nanoparticles consisting of PLGA and PEG diblock copolymers.

Materials and Methods: SiRNA against hTERT catalytic subunit of telomerase was designed and encapsulated with magnetic copolymers using the w/o/w in-water drying method. Transmission electron Microscope (TEM), Fourier transform infrared spectroscopy (FTIR) used for magnetic nanoparticles and copolymers synthesis and Scanning electron Microscope (SEM), was used for siRNA –loaded magnetic nanoparticles modified with PLGA-PEG copolymers. Colorimetric cell viability assay was utilized to evaluate cytotoxicity effects. Quantitative Real-time polymerase chain reaction (Q-PCR) was used to evaluate gene silencing activity in groups.

Result and Discussion: Results showed encapsulated siRNA size less than 80-100 nm, viability assay demonstrated that magnetic copolymers enhanced siRNA delivery in lung cancer cells and data analysis with SPSS showed that the rate of gene expression in equivalent concentrations with magnetic copolymers/ siRNA decreased strongly (P=0.001). The ability of siRNA to knockdown essentially any gene of interest has become a major focus of interest just in recent times in order to identify important therapeutic genes and develop siRNA based treatment.

Keywords: Gene silencing, Encapsulation, SiRNA, Target therapy
Title: Catalase and Polyphenol Oxidase Activity in Some Beta vulgaris Cultivars Under Low Temperature

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Abstract: Introduction: It has been well documented that environmental stress such as low temperature can prevent light and dark reactions of photosynthesis in plants and cause formation of reactive oxygen species (ROS). ROS may cause different damage in plant cell organelles. Plant has two defensive systems, enzymatic and nonenzymatic defensive systems. In this study we assay catalase and polyphenol oxidase activity changes under low temperature to find resistant and sensitive cultivars. Method: Four cultivars of Beta vulgaris (JAM, BR1, IC, 7233) seedlings were grown in growth chamber conditions with 16h light and 25/16 °C day/night temperature and 70% humidity. After six-week we put them under low temperature (2°) for two-day. Then we assay catalase activity (by decreasing absorbance due to oxidation of H2O2) and polyphenol oxidative activity (by increasing absorbance due to formation of purpurogallin) by using spectrophotometric. Results: Our results shows that the catalase activity increase significantly only in Jam cultivar and in other cultivars it had not significant changes and polyphenol oxidase activity had no significant change in all cultivars (sig=0.05). Conclusions: It has been concluded that jam cultivar of Beta vulgaris might be prevented ROS damages by increasing of catalase enzyme activity. The enzyme convert ROS particles to H2O and O2 and prevent its damaging activity. Among tested cultivars the Jam cultivar decrease ROS levels with increasing catalase activity, and other cultivars perhaps with nonenzymatic mechanisms such as increasing Ascorbate, glutathione and ... resistant against ROS.

Keywords: Catalase, Polyphenol Oxidative, Beta vulgaris, Reactive Oxygen Species
Title: Increased expression of the CrkII proto-oncogene in malignant salivary gland tumors and pleomorphic adenoma

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Abstract:

Introduction: Salivary gland carcinomas are among comparatively rare heterogeneous malignancies that show locoregional invasion and distant metastasis. Experimental results support the hypothesis that increased CrkII proto-oncogene is associated with cytokine-induced tumor initiation and progression by altering cell motility signaling pathway. The aim of the study was to assess the CrkII expression in common salivary gland tumors and pleomorphic adenoma.

Method: Immunohistochemical analysis of CrkII expression was performed on paraffin blocks of 64 carcinomas of salivary glands, 10 pleomorphic adenomas and 10 normal salivary glands. Biopsies were subjected to immunostaining with EnVision detection system using monoclonal anti-CrkII. Evaluation of immunoreactivity of CrkII was based on the immunoreaction intensity and percentage of stained tumor cells which were scored semi-quantitatively on a scale with four grades 0 to 3.

Results: Increased expression of CrkII was seen (P=0.005) in all malignant tumors including: mucoepidermoid carcinoma, adenoid cystic carcinoma, acinic cell carcinoma and carcinoma ex pleomorphic adenoma (CaexPA). CrkII expression in pleomorphic adenoma was weak or negative in some biopsies. A weak staining was seen in normal acinar serous cell, while CrkII positive immunostaining reaction was totally absent in normal epithelial duct cells. There was significant positive association between CrkII expression and pathological features of tumor invasiveness.

Conclusions: Increased expression of CrkII and its relation to the invasiveness in carcinomas of salivary gland is consistent with a role for this proto-oncogene in salivary gland tumorigenesis and cancer progression.

Keywords: CrkII, salivary gland carcinoma, immunohistochemistry, invasiveness
Title:
Serum Receptor Activator of Nuclear Factor-k B Ligand, Osteoprotegrin, and Total antioxidant capacity in Tuberculosis.

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Abstract:
Introduction: Tuberculosis (TB) causes stimulation of innate immune cells to produce reactive nitrogen intermediates and reactive oxygen species. A higher level of the free radicals and Inflammation is the main causes of excess generation of oxidative stress and wide variety of damages in patients with TB. Recently the Osteoprotegerin (OPG)/ receptor activator of nuclear factor-kappa B (RANK) / receptor activator of nuclear factor-kappa B ligand (RANKL) system have emerged as an important contributing factor to atherogenesis and osteogenesis. The present study was carried out to evaluate OPG, RANKL, oxidant and antioxidant statues in untreated active pulmonary tuberculosis patients (PTB).

Method: 42 patients with different forms of untreated active PTB admitted to the Research Center for TB and Pulmonary Diseases of Tabriz, Iran together with 45 healthy male subjects as a control group with no history of TB and matched with case for age and sex, were included in this study. Serum levels of OPG, (RANKL) and tumor necrosis factor alpha (TNF-α) were quantified by enzyme-linked immunosorbent assay. Malondialdehyde (MDA), Total antioxidant statues (TA) and lipid profiles were measured by spectrophotometry methods.

Results: Patients with PTB were detected to have significantly higher plasma MDA, TNF-α, OPG, RANKL and lower TAC levels in comparison with healthy controls( p<0.05 all the cases). Conclusions: Low TA and high MDA levels in PTB patients are indicators of oxidative stress in these patients. Evidence of severe oxidative stress and increased RANKL and OPG in our study may induce vascular calcification in PTB patients.

Keywords: pulmonary tuberculosis patients (PTB), Osteoprotegerin (OPG), receptor activator of nuclear factor-kappa B ligand (RANKL) , tumor necrosis factor alpha (TNF-α), total antioxidant statues (TA)
Title:
Influence of magnetization on Physical and chemical properties of irrigation water

Authors:
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Abstract:
Water presence is fundamental for the all life on the planet, because it is nutrient source and the place where chemical reactions are accomplished. Water, being dipolar, can be partly aligned by a magnetic field. The change of the structure of water when exposed to a magnetic field is important in various applications. This article examines the effect of a magnetic field on Physical and chemical properties of water. An alternative magnetic field of 110 mT was used for magnetization of water. IR, FT-IR adsorption spectrum and Raman spectrum, as well as total hardness of water before and after magnetization were achieved. The results showed that total hardness of the magnetized water decreased by 8.3 %, compared with that of the control. Change of Raman spectrum of the magnetized water suggested changes in the distribution and polarization of water molecules but not in constitution of it. This was deduced from an increase in some of the peaks strength, shift in their frequencies, as well as appearance of some additional peaks. Changes in polarization of water molecules and reduction of its hardness open new approaches for a sustainable development in future agricultural management.

Keywords: Water; Magnetic field; Raman spectrum; Total hardness
Title: Osteoprotegrin and renal osteodystrophy in renal failure

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Abstract:

Objective: The aim of the present study was to evaluate serum receptor activator of nuclear factor-κ B ligand (sRANKL), osteoprotegrin (OPG), and intact parathyroid hormone (iPTH) as the main factors for vascular calcification and inflammation in hemodialysis (HD) and renal transplant (RT) patients.

Method: Serum was obtained from 45 stable chronic HD patients and 44 stable RT recipients. Biochemical factors, iPTH, OPG, sRANKL levels were determined by standard methods.

Results: Osteoprotegrin (P = 0.001) and intact parathyroid hormone (P = 0.001) levels in the hemodialysis patients were higher than the renal transplant recipients. Osteoprotegrin had positive correlation with duration of dialysis and age in the hemodialysis (r = 0.88, P = 0.001 and r = 0.34, P = 0.02, respectively) and renal transplant patients (r = 0.92, P = 0.001 and r = 0.46, P = 0.001, respectively).

Conclusion: Hemodialysis patients have higher osteoprotegrin levels than the renal transplant recipients. It may act as a protective factor for renal osteodystrophy or only as a secondary phenomenon of advanced renal failure.

Keywords: Serum receptor activator of nuclear factor-κ B ligand (sRANKL), osteoprotegrin (OPG), intact parathyroid hormone (iPTH), hemodialysis patients (HD), renal transplant patients (RT)
Title:
Protein Formulations Based on Biodegradable Block Copolymers

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Abstract:
The traditional way of delivering a protein drug requires daily, sometimes multiple, injections to achieve its therapeutic effectiveness. To improve patient convenience, sustained release dosage forms have been developed. Recently, many therapeutic proteins have been encapsulated in micro-/nanoparticles made of biodegradable block copolymers of poly (lactic acid) and PEG (PEG/PLA). However, an important issue frequently overlooked is alterations in the drug structure upon its incorporation in such particles. In this study LA37-EO136-LA37 triblock copolymers were employed to prepare a protein delivery system. To assess such alterations, bovine and human insulins, as model proteins, were each incorporated in polymeric micelles and their structural properties compared.
UV-vis spectrophotometric titrations were used to determine the binding affinities of the copolymer to bovine and human insulins. Results indicate that the copolymer displays 3:1 molecular complexes with both types of insulins. The copolymer shows a higher binding affinity to bovine insulin which could be ascribed to the higher hydrophobicity of this protein. Changes in the secondary structure of proteins upon interaction with the copolymer were studied by far-UV CD spectra. At low polymer concentrations, a small decrease in the helical content of insulin is observed. Upon increasing the copolymer concentration, first the secondary structure almost recovers its native form and then there is a significant decrease in helicities of insulin. The results are supported by acrylamide quenching experiments showing reduced accessibility of tyrosines. Upon addition of the copolymer, tyrosines of insulin, located in helices, are exposed to the hydrophobic environment of micelles. Hence, the tyrosine accessibility is reduced.

Keywords: Insulin, Block copolymer, Lactic acid, Micelle.
Title:
Studies on Gemini Surfactants as Membrane Mimetics

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Abstract:
Gemini, or dimeric, surfactants are amphiphilic molecules composed of two hydrocarbon tails and two polar head groups joined together by a spacer. The spacer could be short or long, rigid or flexible. The polar head groups could be cationic, anionic or nonionic. Gemini surfactants are either symmetric or non-symmetric depending on the composition of the two constituting chains. They self-assemble, in aqueous solution, to form supramolecular structures. Gemini surfactants have also remarkably low CMC values compared with the corresponding conventional surfactants of equivalent chain length. It has been shown that the shape and size of the assemblies are influenced by the surfactant composition and the environmental conditions. Furthermore, dimeric surfactants are considerably more surface-active than their conventional analogs. They have possible applications in biotechnology, nanotechnology, gene transfections, analytical separations, antiseptics, skin irritation-free cosmetics, enhanced oil recovery, preparation of high porosity materials, and as paint additives. Thus, gemini surfactants have attracted great attention of various academic and industrial research groups.

In this research, a series of novel cationic gemini surfactants with different compositions were used to prepare nanoparticles as drug delivery vehicles. First, the particles were fabricated in aqueous solution by several methods and the storage stability of the dispersion was investigated. Results showed that in this respect, the thin film method was superior. The work will then characterize some physicochemical properties of the particles to determine whether they could be promising candidates for stable drug carriers.

Keywords: Gemini surfactant, Cationic, Surface active, Thin film, Dispersion
Title:
Analysis of the enzyme aspartate amino transferase (AST), and total salivary protein (TSP) in patients with peptic ulcer

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Abstract:
Peptic ulcer diseases (encompassing gastric ulcer and duodenal ulcer) affect a large portion of the world population and are induced by several factors, including stress, smoking, nutritional deficiencies, and ingestion of non-steroidal anti-inflammatory drugs (NSAIDS) e.g. Aspirin. The pathophysiology of these ulcers involves an imbalance between offensive (acid, pepsin, and Helicobacter pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). Saliva has become an important resource for evaluating physiological and pathological conditions in humans. The use of saliva has many advantages, including the simple and non-invasive method of collection and its easy, low-cost storage. With the addition of modern techniques and chemical instrumentation equipment, there has been an increase in its use for laboratory investigations, applicable for basic and clinical analyses in the fields of medicine and dentistry. In this study the level of enzyme in saliva of patients with peptic ulcers including aspartate aminotransferase, and total protein was detected and compared with healthy persons as control. In practical section, 20 patients with peptic ulcer (10 male and 10 female) and 20 healthy persons as control (10 male and 10 female) were selected. Activity of aspartate amino transferase (AST) and total protein (TSP) were assayed by a spectrophotometric method. The obtained data were analyzed using SPSS software and t-test. Our results showed significant alternation in TSP and ASP of patients with peptic ulcer as compared to healthy control. In fact, activity of AST was significantly increased in salivary fluid of patients with peptic ulcer. The increased level of the enzyme may be explained by considering immunological changes and nutritional changes that occur during the disease course.

Keywords: Salivary diagnosis, Enzyme analysis, Peptic ulcer
Title: Analysis of dinucleotide repeats in intron 1 of PIK3CA gene and its association with colorectal cancer

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Abstract:

Introduction: Colorectal cancer is cancer that starts in the colon or the rectum that are parts of the digestive system. Before a cancer develops, a growth of tissue usually begins as a non-cancerous polyp on the inner lining of the colon or rectum. A tumor is abnormal tissue and can be benign or malignant. The majority of colon cancers are derived from genetic changes that occur within the epithelial cells of the bowel wall. Mutation in PIK3CA gene play an important role in colorectal carcinogenesis. Bioinformatic studies of PIK3CA gene show a region with GT sequence repeats in the intron 1 of the gene. The polymorphism and the association of the GT microsatellite with colorectal cancer have been studied before. The aim of this study is to analyze the GT polymorphism in the intron 1 of PIK3CA gene and specify its association with colorectal cancer.

Method: Genomic DNA is extracted from the blood of patients with colorectal cancer and after designing primers, the intron 1 of PIK3CA gene is amplified by PCR. Finally the length of the repetition sequence is analyzed by polyacrylamid gel.

Results: So far we have observed four alleles with different numbers of GT dinucleotide repeats in the intron 1 of PIK3CA gene in patients and its relationship with colorectal cancer is under investigation.

Conclusion: Characterizing the number of GT repeats and its association with colorectal cancer can help us in diagnosing people susceptible to colorectal cancer and can also have therapeutic purposes.

Keywords: Colorectal cancer, PIK3CA gene, Polymorphism.
Title:
Viscosity B-coefficients of Neutral Salts of the Hofmeister Series and their roles in interpreting the enzyme stabilization

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Abstract:
Introduction: The viscosity B-coefficients of salts (as well as their ions) allow us to compare the ability of salts in stabilizing of proteins that are valuable clues for comprehending the protein unfolding. Stability of the horse liver alcohol dehydrogenase correlates well with the Hofmeister series in terms of the salt's kosmotropic/chaotropic properties, which are assessed by the Jones–Dole viscosity B coefficients (B\(^+\) for cations and B\(-\) for anions). For the purpose of this study, stability of horse liver alcohol dehydrogenase are assessed in presence of high concentration of salts (1-4 M) containing ions that have different viscosity B coefficients.

Method: In this study, the effects of Hofmeister salts on thermal stability of enzyme in two temperatures (50 and 60 °C) were investigated.

Results: In our experiments, all the stability data correlated similarly well with both the B\(-\) and the (B\(-\)– B\(^+\)) values of the salts tested. This suggests that in terms of affecting the enzyme stability, the anion of the salt plays a more predominant role relative to its cation. Acetate anion may induce greater thermal stability (half-life increased from 300 to 560 min at 60 °C).

Conclusion: The stability study of alcohol dehydrogenase offers another example of more kosmotropic anions and chaotropic cations favoring higher enzyme stability.

Keywords: Key words: Horse liver alcohol dehydrogenase, thermal stability, Hofmeister Series, Kosmotrope and Chaotrope
Title: A simulation study of AQP5 and calculation of energies between groups of atoms by using QM/MM and Monte Carlo methods

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Abstract: Aquaporins (AQP5) are proteins embedded in the cell membrane that regulate the flow of water. More than 10 different mammalian AQP5s have been identified. Human AQP5 facilitates the transport of water across plasma membrane and has been identified within cells of the stomach, duodenum, pancreas, airways, lungs, salivary glands, sweat glands, eyes, lacrimal glands, and inner ear. In the present work the quantum mechanics/molecular mechanics (QM/MM) and Monte Carlo methods were used to study and calculate the potential, kinetic and total energies of 1500 first atoms when this protein has 4000 atoms. The divided 1000 atoms in 100 atoms groups were studied and the energies were calculated and compared. Method: In this research the QM/MM and Monte Carlo programs were used.

Results: the calculation on the 1500 atoms of AQP5 shows that the maximum amounts of potential and total energies are belong to a group of isolated atoms located in 100 first atoms. Also the minimum energy is found in a group located in the 600-700 atoms space. The kinetic energy of both groups was calculated and it was the same.

Keywords: AQP5, QM/MM, Monte carlo
Title:
A novel method for determination of folic acid in fortified wheat flours and breads by High Performance Liquid Chromatography (HPLC)

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Abstract:
Introduction:
Folate has been identified as one of the most important water-soluble B vitamins for normal metabolic function and it is essential for normal human cell division and cell growth. Since humans cannot synthesize folates, they must obtain from dietary sources. The most important dietary sources of folates are fortified foods, and cereal products with folate content of 50-200 µg/100 g. In Iran the majority of people consumed bread as an ideal food, because of the cheapest and availability. The objective of this study was to describe a method for determination of folic acid in wheat flours and breads.

Method:
The method consists of several procedures, including an extraction technique, oxidation of folic acid to increase fluorescent properties and a modified HPLC with fluorescence detection method. Thirty enriched flours and thirty bread samples were purchased from the local baker markets in the city of Urmia.

Results:
The standard curve for folic acid passed through the origin and was linear over the range 0.1-8 µg/L. The peak of folic acid appeared as sharp with retention times of 1.12 minutes. The accuracy of the method ranges between 99.2% and 103.7% for intra-day analysis and 98.8% and 106.9% for inter-days analysis. The limit of detection for folic acid was 0.03µg/L. The mean concentration of folic acid was 102 µg and 85 µg per 100 gram of flour and bread respectively.

Conclusions:
This work is a preferred method for folic acid analysis because it is rugged, fast, specific and sensitive. Estimation of folic acid in wheat flour was less than those based on calculations as done for enriched flours. Samples were 16.67% decrease of added folic acid from flour to bread stage.

Keywords: Folic acid, HPLC, Flour, Bread
Title:
Adaptation of recombinat human Growth Hormone producing CHO cells to serum-free suspension culture

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Abstract:
Introduction: The large-scale production of biopharmaceuticals, such as recombinant human Growth Hormone (rhGH), commonly requires the use of serum-free medium, for both biosafety and economic reasons. Chinese Hamster Ovary (CHO) cells are one of the most frequently used cell lines for the expression of recombinant proteins. CHO cells used for large-scale production of recombinant proteins are typically grown in suspension cultures using animal serum-supplemented medium. However, animal serum presents several well-documented problems for biopharmaceutical manufactures of therapeutic agents such as interference in protein purification and danger of contamination with pathogens. as serum is such an essential supplement of the growth medium for most mammalian cells, its removal demands a very time-consuming process of cell adaptation. In this process, cells are usually subjected to a gradual decrease of serum concentration in the medium. The purpose of this study was to adopt rhGH producing CHO cells to serum-free suspension culture for large-scale production and decrease concerns surrounding the use of animal-derived components in the production of therapeutic proteins.

Method: CHO cells were adapted to grow in suspension serum-free medium (SFM) culture using the sequential adaptation method and then cells were transferred to the roller bottle for large-scale production. samples were taken every 48 hours and then cell viability and rhGH production was assessed using flow cytometry, ELISA, western blotting and dot blotting.

Results: by adaptation of SFM, CHO cells changed from monolayer to suspension culture successfully. The suspension CHO cells produced comparable recombinant protein to the complete medium containing medium.

Conclusion: The suspension adaptation of CHO cells in serum free medium can be achieved successfully without dramatic loss in protein production. This has a significant effect on the downstream purification process.

Keywords: CHO Cells, Serum Free Medium, ELISA, western blotting, recombinant human Growth Hormone
Title:
Spatio-Temporal modeling and prediction of Tuberculosis incidence rate in Khorasan Razavi province (Kashmar, Khalilabad and Bardeskan cities)

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Abstract:
The past two decades have witnessed an increasing interest in the use of space-time models for a wide range of environmental and epidemiological problems. The spatio-temporal epidemiology is one of the most important tools for epidemiologists to detect, monitor and predict public health disease patterns. Nowadays, tuberculosis (TB) infectious diseases caused by the Mycobacterium are very concerned with it and its association to several other diseases and factors. Each year, tuberculosis kills about three million people in the world. The purpose of this study is to determine if there are spatiotemporal tuberculosis incidence model in Kashmar, Khalilabad and Bardeskan which are cities of Khorasan Razavi province (Iran). The presented case study is based on the notification of new tuberculosis cases (disease incidence), between 2006 and 2012. In methodological terms, the spatio-temporal kriging (best spatio-temporal linear prediction) statistic, used to identify spatio-temporal modeling and prediction of TB incidence for two next years (2013-2014). For this case study, the number of new notified cases of TB, per sub-district and per year (2006–2012) was available. In terms of spatiotemporal modeling of tuberculosis disease, the proposed methodology allowed the identification of critical areas with critical incidence rate. Results indicate three critical regions with maximum TB incidence rate in each cities for two next years. Results indicate that in these critical regions must perform prophylactic actions to decrease TB incidence rate on next years.

Keywords: Spatio-Temporal, modeling, Tuberculosis
Title: Study on interaction between Gαq and β-Catenin signaling pathways in HEK-293T cells

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Abstract: Introduction: The Wnt/β-Catenin signaling pathway regulates multiple cellular processes and therefore its abnormal activation has been observed in various human cancers. We and others have got some evidence that heterotrimeric G-proteins regulate Wnt signaling. Our previous results have shown that activation of Gαq in HEK293T cells increases cellular β-Catenin protein levels. Here we have investigated the biological outcome of Gαq-mediated cellular accumulation of β-Catenin by measuring the expression of several β-Catenin-target genes like cyclin D1, c-myc, and fgf-20.

Methods: HEK-293T cells were grown under standard conditions and then were transfected with the Gαq-encoding plasmid or treated with thrombin and carbachol, two agonists of the Gαq signaling. Cellular β-Catenin protein levels and its intracellular localization were analyzed by western blotting and immunofluorescence microscopy assays. Gene expression was assayed by reverse transcriptase-PCR and (or) real time-PCR.

Results: Thrombin or carbachol treatment of HEK-293T cells led to an approximate two-fold increase in cellular β-Catenin protein levels, although the target gene transcription was not enhanced more than 1.5-fold. Consistently, transfection of cells with low amounts of the Gαq-encoding plasmid led to about 2-fold increase in transcription of the selected target genes. Interestingly, we found out that overactivation of Gαq which normally produces high cellular levels of β-Catenin, has a negative effect on β-Catenin-target gene expression. Our laboratory is currently investigating this issue.

Conclusions: The current results suggest that although upregulation of β-Catenin protein levels by Gαq is biologically relevant, the interaction between these two signaling pathways is tightly regulated.

Keywords: Keywords: β-Catenin, Gαq, HEK-293T
Title: Artemin prevents heat-induced aggregation of β-lactoglobulin

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Abstract: Encysted embryos of Artemia are exceptionally resistant to severe environmental stress. This resistance is thought to depend in part on the existence of a protein termed artemin. A recent research has indicated that artemin influences on the folding pathway of bovine β-lactoglobulin (BLG). In this study, the effect of artemin has been evaluated on aggregation of BLG used as protein substrate for study of the protein aggregation. At high temperatures, exposure of buried inner hydrophobic groups and the thiol group of BLG causes to the formation of disulfide-linked aggregates.

Method: In the present study, recombinant artemin, previously isolated and cloned from encysted embryos of Artemia urmiana from the Urmia Lake, IRAN, was expressed in E. coli and then purified. Aggregation of BLG was studied by SDS-PAGE and turbidity measurement. BLG was solved in 5 mM acetate buffer, pH 5.0 containing 60 mM NaCl and heated at 80 °C for 20 min. Then, the turbidity of the samples was determined by measuring their absorbance at 600 nm. SDS-PAGE of native and heated BLG was also performed for quantitative analysis of monomer, dimer, trimer and aggregated forms of the protein.

Results: The results indicate that, artemin prevents BLG aggregation in a concentration-dependent manner. The extent of turbidity was reduced approximately by eight and four folds in the presence of 5 and 15 µg ml-1 of artemin. In addition, SDS-PAGE indicated intensity of bands for dimer forms reduces upon interaction of BLG with artemin.

Conclusion: Artemin thermal aggregation of BLG probably by preventing exposure of buried inner hydrophobic groups.

Keywords: Artemin, β-lactoglobulin, Aggregation, Chaperone
Title: Modeling and theoretical studying of the sequence dependence of nucleosome sliding in genome

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Abstract:

Introduction: DNA in chromatin is wrapped around histone octamer to form nucleosome complexes. The spatial accessibility of nucleosome protected sequences differs dramatically from their nucleosome free counterparts, so that nucleosome positioning and distribution may affect DNA-related cellular processes such as DNA replication, repair, transcription, and recombination. Nucleosomes need therefore to be repositioned. There are two ways of repositioning; (i) sliding nucleosomes along the DNA, and (ii) removing (ejecting) histone octamers from DNA.

Method: we are trying to propose a dynamical model of nucleosome sliding. It has been shown that affinity of wrapping around histone octamer is sequence dependent. We have developed a theoretical model; the position of histone octamers on DNA as a function of DNA sequence. We are investigating nucleosome sliding and occupancy probability through Monte Carlo simulation.

Results: we demonstrated that sequence coding has an important role on nucleosome sliding and occupancy probability.

Conclusions: We have developed a new method for describing the relationship between DNA sequence and nucleosome affinity.

Keywords: Chromatin, Histone, Nucleosome, Sliding
Title:
Introduction of disulfide bond into mnemiopsin photoprotein and its effect on conformational stability of the protein

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Abstract:
Introduction: Bioluminescence is the process of converting chemical energy into the energy of visible light by living organism. It has found many applications in different fields of biological sciences. All reported Ca\(^{2+}\)-regulated photoproteins have only one subunit of about 21.4-27.5 kDa, and they are thought to contain a common organic substrate, coelenterazine, and molecular oxygen bound in the form of a complex. The mnemiopsin system is similar to aequorin from the jellyfish Aequorea, except that it can be light-inactivated. It has been proposed that the stability of a protein may increase through the introduction of a disulfide bridge that decreases the configurational entropy of unfolding. According to bioinformatic studies, it is expected that introduction of disulfide bridge into mnemiopsin increase its stability and also sensitivity to light. Here, new cysteine residues for formation of disulfide bridge has been introduced into mnemiopsin protein.

Method: The changes of single and double mutation of (C163-C170) and (C163A-C170A) have been applied by site-directed mutagenesis. pET28a(+) containing native and mutant mnemiopsin were sequenced using an automatic sequencer by T7 promoter and T7 terminator universal primer. Native and mutant mnemiopsin were expressed in BL21(DE3) cells and purification of His\(_6\) tagged fusion proteins were performed by Ni-NTA spin column. Purified protein was analyzed by SDS-PAGE.

Results: Introduction of single and double disulfide bridge influence on conformational stability of mutant photoprotein.

Conclusion: Introduction of disulfide bridge is a strategy that change stability of mnemiopsin.

Keywords: Photoprotein, Mnemiopsin, Biolumincence, Disulfide bridge
Title:
Fluorimetry studies on the trypsin in the presence of ethanol.

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Abstract:

Introduction: Trypsin (EC 3.4.21.4), a member of large family of serine proteinase, that hydrolyzes the Special peptide bonds involving lysine and arginine residues and play major roles in biological process including digestion, activation of zymogens chymotrypsin and other enzymes. It has a molecular mass of 24000 Da and consist of 223 amino acid residues. In this study, the fluorescence emission spectra of trypsin at various concentrations of organic solvent(ethanol) was measured.

Method: The fluorescence emission spectra of tryptophan residue in trypsin –ethanol were measured using a fluorescence spectroscopy (shemadzu RF530Ipe ) at pH 8 at 30 °c using sodium phosphate buffer with the emission range of 300-450nm. Measurement were carried using 0.5mg/ml concentration of trypsin and 5-25% ethanol concentrations.

Results: The study showed with increase of the ethanol concentration, the fluorescence emission is increased.

Conclusions: Fluorescence is an excellent probe to investigation conformational changes. The result of the present study showed that in the presence of ethanol, fluorescence emission of trypsin increased indicating the structure of enzyme changed and tryptophan is transfered to the more hydrophobic region or keep out charged groups.

Keywords: Trypsin, Ethanol, Fluorescence spectroscopy, Emission, Residue
Title:
Cationized human serum albumin based nanostructures for metal chelation therapy in neurodegenerative disorders

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Abstract:
Introduction: Recent attention has focused on the accumulation of iron in neurodegenerative disorders and the strategy of lowering brain metal ions through the administration of chelators has great potential in their treatment. Cationized human serum albumin (CHSA) which is able to pass through blood brain barrier via adsorptive mediated transcytosis was used here to prepare conjugated nanostructures of a chelator agent (deferasirox) to target iron deposition in brain. Methods: CHSA was prepared by ethylenediamine in the presence of 1-ethyl-3-(dimethylaminopropyl)-carbodiimide hydrochloride (EDC) in an ice bath. The result was concentrated and purified by centrifugal filter devices and extensive dialysis. The resulted CHSA was derivatized by deferasirox molecules. For this purpose, deferasirox (18 umoles) carboxylic groups were activated by EDC and N-Hydroxysulfosuccinimide (SNHS) and were further used to derivatize CHSA. The resulted conjugates were concentrated and purified. The conjugated CHSA was characterized by gel permeation chromatography (GPC). Size and size distribution of the resulted conjugates were analyzed by Zetasizer Nano ZS. Results: The conjugation ratio was between 6 to 18 deferasirox moles per each CHSA mole. The conjugated proteins were analyzed by GPC and an increase in the size of conjugated proteins was observed. 97% of the nanostructures had a size of about 50 nm. The mean particle diameter was 165 nm with a particle size distribution of 0.315. Conclusion: A simple method was developed for the preparation of water soluble nano structured conjugates of deferasirox by carbodiimide coupling mechanism which has the potential of brain targeting for neurodegenerative disorders.

Keywords: metal chelation, Alzheimer’s disease, iron, cationized human serum albumin, conjugation
Title: Molecular cloning of a new laccase gene from Bacillus pumilus

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Abstract: Introduction: Laccases (benzenediol oxygen oxidoreductase; EC 1.10.3.2) are multicopper oxidases capable of oxidising a broad range of aromatic compounds. They are particularly promising enzymes for biotechnology and bioremediation purposes that are distributed among plants, fungi and bacteria. To date, only a few bacterial laccases have been characterized and found to be highly active and stable at high temperatures and pH values. Therefore, in this study we have cloned a gene encoding a laccase from Bacillus Pumillus that was previously isolated.

Methods: B. pumilus was grown in nutrient broth medium at 37 °C and the genomic DNA was extracted. The gene encoding laccase was amplified using designed specific primers. The PCR amplicon was digested with Xho1 and Nde1 and ligated into the expression vector pET28a. The resulting recombinant plasmid was then transformed into Escherichia coli BL21 (DE3) for expression.

Results: The cloning process was verified by double digestion and sequencing analysis. Nucleotide sequencing revealed an open reading frame of 1533 bp encoding 510 amino acid residues.

Conclusions: Bacterial expression system has the benefit of being relatively simple compared with other expression systems such as fungi. High level production of recombinant laccase in E. coli BL21 (DE3) and a simple purification of the expressed His-tagged protein make the enzyme suitable candidate in various fields of biotechnology and bioremediation applications.

Keywords: Laccase; Bacillus pumilus; Cloning; Bioremediation
Title:
A comparative study on the interaction of water soluble zinc porphyrazine with G and C-rich strands and the complete c-MYC silencer element

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Abstract:
Introduction: c-MYC gene plays an important role in the regulation of cell proliferation and growth and it is overexpressed in a wide variety of human cancers. Around 90% of c-MYC transcription is controlled by the nuclease-hypersensitive element III1 (NHE III1), whose 27nt purine-rich strand has the ability to form G-quadruplex structure under physiological conditions. c-MYC DNA is an attractive target for drug design, especially for cancer chemotherapy. While previous studies have focused on the G-quadruplex in the c-MYC promoter. Here the interaction of water-soluble tetrapyridinoporphyrazinatozinc(II) with 27nt G-rich strand (G/c-MYC) and its equimolar mixture with the complementary sequence (GC/c-MYC) as well as related C-rich oligonucleotide (C/c-MYC) has been investigated.

Method: Circular dichroism (CD) was used to recognize the structure of three forms of oligonucleotides and the effect of the complex binding on their conformation. Binding mode and constant were determined by spectrophotometric and spectrofluorometric techniques.

Results: CD measurements of the G-rich and C-rich 27-mer oligonucleotides in 150 mM KCl, pH 7 demonstrated a spectral signature consistent with G-quadruplex DNA and i-motif for G/c-MYC and C/c-MYC respectively. Furthermore, CD spectrum indicated dominant structure of GC/c-MYC in the presence of K+ ion is double helix. Absorption spectroscopy indicated that the complex binding is a two-step process; in the first step, small red shift and hypochromicity and in the second step large red shift and hyperchromicity were observed in the Soret band. Emission spectra of zinc porphyrazine-thiazole orange complex were quenched on addition of three types of DNA.

Conclusion: zinc porphyrazine binds with different affinity to duplex, quadruplex and i-motif forms of c-MYC gene.

Keywords: Porphyrazine, c-MYC promoter, DNA, Binding
Title:
Dendrosome-based delivery of siRNA against Hsp27 in human hepatoma cells

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Abstract:

Introduction: Lipofectamine 2000 has been used successfully to transfect short interfering RNAs (siRNA) into mammalian cells for RNA interference (RNAi) studies but Experiments showed that lipofectamin has toxic effects on target cells which necessitates using a safer carrier such as dendrosome. dendrosomes are neutral, biodegradable, covalent or selfassembled, hyperbranched, spheroidal nano-particles with size ranging from 15 to 100nm that provides an efficient mean for gene delivery into various kinds of cells such as human hepatoma. In this experiment, We've used dendrosome As a safe carrier to transfact short interfering RNAs (siRNA) into human hepatoma cell.

Methods: study was conducted on Huh7 cells. Under sterile conditions 4mL of the culture medium (DMEM) and 10% FBS was pipetted into a 25cm² flask (37 °C). After 72 hours , Cells were cultured in a plates 12 wells. and After 24 hours , Dendrosome –siRNA and lipofectamin –siRNA Complexes were prepared and added to each well. At time intervals of 24 and 48 and 72 hours, RNA was extracted and examined by RT -PCR .

Results: The results indicate that siRNA is transferred into huh-7 cell and Expression levels of hsp 27 are decreased.

Conclusions: dendrosomes is neutral, biodegradable, covalent or selfassembled, hyperbranched, spheroidal nano-particles that can be a good alternative to other carriers such as Lipofectamin in the gene delivery.

Keywords: Lipofectamine 2000, short interfering RNAs, dendrosomes, RT –PCR
Title:
Effect of ultrasound on growth and membrane integrity of suspension-cultured parsley cells

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Abstract:
The perception of mechanical stimuli is crucial to the survival of plant cells and is proposed to be driven by a plant-specific mechanosensory network, similar to that of the animal systems. This sensory network may account for the perception of numerous mechanical signals including gravitropic, turgor pressure, and sound. Ultrasound is a physical tool for manipulation of plant cells and is usually used to improve extraction procedure of secondary metabolites. Recent researches however, have noted that perception of ultrasound by the plant cells may be beneficial to them and result in alteration of certain metabolic pathways, rather than merely change of the membrane permeability. In the present research, suspension-cultured parsley cells were treated with ultrasound at 29 kHz with the power of 455 mW/cm², for 10, 20, and 40 min. Viability of the cells was assessed with Evans Blue (aqueous, 0.05% w/v) and changes of membrane permeability was evaluated by measuring the lipid peroxidation rate and electrolyte conductivity of the cells. According to the results exposure to ultrasound for 10 min did not change neither the growth nor viability of the cells nor lipid peroxidation of their membranes. Exposure for longer periods however decreased the growth and increased pH but had no effect on EC. The results suggest that ultrasound affect the function of ion channels in membrane of parsley cells.

Keywords: mechanoperception, membrane integrity, parsley cells, ultrasound
Title: Optimization of Production and Purification of KOD DNA Polymerase in Escherichia coli

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Abstract: Introduction: The PCR is an indispensable method in biotechnology. One of the limitations of PCR is the size of the amplified DNA product. The basis for this constraint is the error rate and low proccessivity and elongation rate of different thermostable DNA polymerases. However, if an efficient thermostable PCR enzyme with higher accuracy, elongation velocity and proccessivity such as KOD DNA polymerase is used an increased yield of a high fidelity long_range PCR products can be produced. The aim of this study was to optimize of the production and purification of a functional KOD DNA polymerase.

Method: We have purchased a pET17b plasmid that had KOD DNA polymerase gene in multiple cloning site and transform it in the expressing strain BL21(DE3) containing the pLysE or pLysS plasmid. Production of the 90-kDa protein was induced by different concentrations of IPTG. Purification were performed by heating, ammonium sulfate precipitation and chromatography with DEAE-sepharose. Purified protein was identified by SDS-PAGE and AgNO3 staining. The activity of the obtained enzyme was measured by comparing the intensities of the produced DNA bands in PCR reactions.

Results: The correct framing of the gene and its orientation were analyzed by digestion and colony PCR. The plasmid was stable in BL21(DE3) containing the pLysS plasmid. Addition of glycerol, Triton X-100, Tween 20, dextrose and protease inhibitor cocktail increased enzyme stability.

Conclusions: We have found that pET17b expression vector is toxic or unstable in the expressing strain BL21(DE3), even in the absence of induction. However BL21(DE3) containing the pLysS plasmid suppresses expression prior to induction and was able to produce greater amount of enzyme after induction by IPTG.

Keywords: PCR, KOD DNA polymerase, BL21(DE3)pLysS, Protein purification, Chromatography
Title: Comparative modeling of Bacillus cereus Hemolysin for virtual screening of potential inhibitors of this toxin

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Abstract:
Introduction: Hemolysin is a Sulfhydryl-activated toxin with hemolytic activity against host red blood cells. It also Causes cytolysis by forming pores in cholesterol containing host membranes. Bacillus cereus's hemolysin binds to target membranes, the protein undergoes a major conformation change, leading to its insertion in the host membrane and formation of an oligomeric pore complex. This toxin is a potential target for the therapy of some infectious diseases; therefore by virtual screening of potential inhibitors of this toxin and finding the right inhibitor, therapeutic purposes can be achieved.

Methods: In the current study, a virtual screening by docking was performed among 958 compounds from ZINC library to find a specific inhibitor for Bacillus cereus's Hemolysin. For this point, Modeller v9.11 was used to predict the 3D structure of Hemolysin. Predicted model regarding energy level minimized using UCSF chimera candidate version1.7. Final predicted model was evaluated based on Q-mean, PROCHECK and Prosa score. Molegro virtual docker with its template docking algorithm and MolDock [GRID] scoring function was used to measure the binding affinity of the found inhibitor to the active site of the model.

Results: A search in ZINC database resulted in finding a theoretically efficient inhibitor with high binding affinity to Hemolysin.

Conclusion: Results suggest that the found inhibitor is capable of binding with high affinity to the toxin for blocking cytolysis; therefore therapeutic purposes can be considered.

Keywords: Bacillus cereus, Hemolysin, Docking, Inhibitor, Virtual screening
Title:
Bioinformatic study of using class II restriction enzyme in VEGF-111 cDNA synthesizing

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Abstract:
Introduction: Complementary DNA (cDNA) is often used in gene cloning as gene probes or in creation of a cDNA library. Some various methods were used for cDNA synthesizing and we used a method to create a design for VEGF-111 cDNA synthesizing based upon the use of class II restriction enzyme. VEGF111 is a new proteolysis-resistant splice variant of VEGF-A which is not sensitive to degradation by serine proteases. VEGF encoded by a gene that contains eight exons and VEGF111 isoform consist of exons 1-4 and 8. Splicing is required for rapid and efficient mRNA export from nucleus to cytoplasm. So we decided to insert intron 4-5 of VEGF gene between exon 4 and 8 and designed reverse primer which is used to amplify the intron 4-5 in a way that containing 18 nucleotide of 8 exon.

Material and Methods: First primer pair designed for amplification of exon 1, 2, 3 and 4 on breast tumor cDNA and second primer pair designed for Intron 4-5 amplification on genomic DNA. We chose Eco31I as a II restriction enzyme and designed the reverse and forward primer of first and second primer pair in a way that containing a specific recognition sequence of Eco31I.

Results: Two described fragments were amplified by specific reverse and forward primers and result in 415 and 395 bp fragments. After digestion of fragments with Eco31I and ligation of two fragments, a 810 bp band was seen on gel agarose.

Conclusions: This approach is simple, highly efficient, less error prone and cost-effective and can also be used to fuse different PCR-fragments from distinct gene to create a chimeric gene.

Keywords: cDNA synthesizing, VEGF-111, class II restriction enzyme, Eco31I
Title: Peptide nucleic acids (PNAs) and biosensor technology

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d Author: Dr. Hadi Valizadeh, Professor of pharmaceutics, Tabriz university of medical science

Abstract: Introduction: Nowadays, the importance of monitoring and regulating many different parameters in areas such as clinical diagnosis and drug development is increasing. Therefore, there is a need for available reliable analytical devices, which enable to perform quick and accurate analysis. One of the ways is to use proper designed biosensor. A biosensor is a bio-analytical device that consists of a bioreceptor and a transducer. Biosensors can be classified either by their bioreceptor or their transducer type, such as DNA and PNA biosensors. Synthetic molecules such as peptide nucleic acid (PNA) that can bind with high sequence specificity to a chosen target in a gene sequence are very interest in medicinal and biotechnological contexts. They are DNA mimic in which the sugar phosphate backbone of natural nucleic acid has been replaced by a synthetic peptide backbone consisting of repeating N-(2-amino-ethyl)-glycine units linked by amide bonds. This unique structure gives PNAs the capacity to hybridize to high affinity and specificity to complementary of DNA or RNA and present considerable promise for DNA diagnostics. Conclusion: The unique structural and recognition feature of PNA has stimulated research on the hybridization process and its diagnostic applications. PNA represent great advantages, in comparison with oligonucleotide probes, such as faster, stable and more reliable analytical processes and has led to promising developments in many areas of biotechnology. Recent efforts including modification of backbone and the development of novel base analog can provide further applications of this molecule.

Keywords: Biosensors, DNA and PNA biosensors
Title:
Kinetics and structural studies on the chicken egg white lysozyme in the presence of Fe3O4 magnetic nanoparticle

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Abstract:
Recently, there is very interest in developing magnetic nanoparticles as new materials for biomedical functions like magnetic resonance imaging, hyperthermia for biomedical tumor treatment, cell labeling and sorting, DNA separation and drug delivery. The binding of magnetic nanoparticles to protein is a widespread phenomenon and plays a particularly important role in the activity of an enzyme. In this study the turbidimetric assay (activity) of lysozyme followed by the decreased optical density of a turbid cell suspension (about 0.3 mg/ml Micrococcus lysodeikticus) photometrically at 450 nm. Effect of Fe3O4 magnetic nanoparticle on lysozyme was investigated by UV-Vis spectrophotometry at pH 7.25 at 35°C using sodium phosphate buffer. It was found that by increasing of nano-Fe3O4, the rate of Micrococcus lysodeikticus lyses (lysozyme activity) will be increased. Also, the thermal stability of lysozyme was surveyed in the presence of nano-Fe3O4 over the temperature range (293-363) °K in sodium phosphate buffer and pH 7.25. Results indicated that thermal stability of lysozyme will be increased in the presence of this nanoparticle. Lysozyme consists of two domains: a α-domain with helical structure and a β-domain with predominantly β-sheet, separated by the active cleft. The cleft between the two domains includes the binding site for the substrate. It has been reported that the presence of nano-Fe3O4 can increase the β-sheet and α-helix contents. On the contrary, the contents of γ-random coil and T-turns will be reduced in the presence of nano-Fe3O4. So we conclude that the presence of this nanoparticle can increase the activity of lysozyme

Keywords: Lysozyme, Magnetic nanoparticles, Spectrophotometry, Activity, Protein
Title:
Effect of mercury chlorid on aquaporin activity in green alga Dunaliella salina

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Abstract:
Introduction: *Dunaliella* is a unicellular green algae which do not possess a rigid cell-wall. They respond to changes in osmotic potential of medium by rapid alterations in cell volume by release or uptake the water in order to adjustment of internal osmotic potential. Mercury ions are reported to inhibit water movement by inhibition of some water channels (aquaporin). It seems *Dunaliella* cells are able pass large amount of water cross their membrane via aquaporin in response to osmotic stress. In this study, in order to preliminary study of existence of aquaporin in *Dunaliella*, the effect of inhibitor (HgCl₂) of aquaporin was studied.

Method: The *Dunaliella* cells were cultured in the culture medium containing 1.5M NaCl. The concentration of zero, 5, 10, 25, 50, 85, 130 µM HgCl₂ were used. Then the best concentration of inhibitor was applied and then osmotic shock of 1.5M to 2.5 and 1.5 to 1.0M NaCl was performed. The activity of aquaporins was monitored by cell volume shape and changes using light microscopy.

Results: Results showed that the aquaporins in *Dunaliella salina* are sensitive to the HgCl₂ and the best concentration for inhibition of water movement and blocking the cell volume changes is about 25 to 50 µM, while the cells are alive. In addition, 10mM of mercaptoethanol are able to reverse the alteration in cell volume.

Conclusions: Large amount of water can move through membrane of *Dunaliella* cells via aquaporins. This able the cells to respond to osmotic changes of medium by rapid changes in water content and cell volume.

Keywords: Dunaliella, mercury cholorid, aquaporin.
Title: MD Simulation Study of F-actin Assembly Polymorphism in the Presence of Cross-linking Proteins

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Abstract:

Introduction: The assemblies of filamentous-actin (F-actin) are key components of the eukaryotic cytoskeleton and are generally used for mechanical support including cell motility and adhesion, cell division and help to maintain shape of cells. F-actins are biological rod-like polyelectrolytes that are expected to repel each other. The assembly and organization of F-actin in *in vivo* condition is controlled by varying cross-linking protein. In general, there are two types of cross-linkers which cross-link actin into polymorphisms of bundle and network phases. In this study we aim to study the dependence of the assembly formed by F-actins and the properties of linker proteins such as their lengths and electrostatic charges.

Method: To understand the mechanism for cross-linker-induced F-actin assembly we carry out a molecular dynamics (MD) simulation study. To run MD simulation we used ESPResSo that is a simulation package for the research on soft matter systems. We present a coarse-grained model for the system of actin filaments and cross-linkers (using the 4-sphere model for the G-actin monomer and 2-sphere model for cross-linkers).

Results: After equilibrium of F-actin system, we observed that actin filaments attract each other under the influence of cross-linker. F-actin can form various structures as there are different parameters, including the cross-linker concentration and the properties of cross-linker itself such as their lengths and electrostatic charges. We observe that changing the charge of linkers, change the structures formed by actin filaments and their stability.

Conclusions: MD simulation is a powerful tool to reveal the role of different controlling parameters in the F-actin assembly. Having knowledge about the dominating parameters in the different structures of F-actins and their stability, shed light on how to control the stability of F-actin assemblies formed in some diseases such as airway infections in cystic fibrosis.

Keywords: F-actin, Cross-linker, Polyelectrolyte, Assembly, Bundle and Network phases and MD simulation.
Title:
The analysis of Interferon beta production Improvement by site directed mutagenesis

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Abstract:
Introduction: IFNs discovered in 1957 and the term of interferon originally described the biological activity of soluble substance which “interfered” with viral replication. IFNs are proteins produced by cells in response to antigenic stimulation like viral RNA, bacterial product or tumor proteins. There are three classes of interferon base on the origin of the cells including IFN alpha, IFN beta and IFN gamma. IFN beta has pleotropic effects, including antiviral, antitumor and anti-inflammation. Interferon beta used as a medicine in autoimmune disease like MS and virus infection.

Material and Methods: In order to increase mRNA stability, improve translation and optimization of interferon beta production three recombinant constructs designed by genetic engineering methods. These three constructs have specific mutagenesis including deletion of 53 nucleotide of 3′-UTR, deletion of 18 nucleotides of 3′-UTR by mega primer PCR and finally two nucleotides of Kozak sequence was changed by Soeing PCR due to make it conserve sequence. Recombinant interferon was inserted into the pSVM- dhfr -plasmid. pSVM- dhfr Plasmid transferred to the CHO-dhfr- cell line by using Lipofectamine kit.

Results: This modification will eventually increase the interferon expression in CHO cell line and will confirm by Real Time PCR method. After analysis the best Construct will be chosen for more researches and insert to plasmid for protein purification.

Key words: interferon beta, DHFR-CHO cell line, pSVM-DHFR plasmid

Keywords: interferon beta, DHFR-CHO cell line, pSVM-DHFR plasmid
Title:
Magnetic water application for improving maize (Zea mays L.) crop production

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Abstract:
Water molecules can be affected by magnetic fields due to their bipolar character. In the present study an experimental maize field was irrigated by tap water which was magnetized by passing through a locally designed alternative magnetic field generating apparatus (110 mT). The plants were irrigated by the magnetized water from sowing to the end period of generative stage. The effects of magnetized water on the content of ferritin, iron and calcium of seeds were evaluated by the method of ELISA and semi-quantitative XRF, respectively. Treatment with magnetic water increased the ferritin, iron and calcium contents of kernels (23%, 135% and 84%, respectively), compared with the control groups. Therefore, it appears that utilization of magnetized water can led to improve quality of maize crop. Extensive further research using magnetic water treatment on different crops may approve this as a promising technique for agricultural improvements.

Keywords: Calcium; Ferritin; Iron; Magnetic water; Maize.
Title: Designing a Genetic Construct Expressing EGFP Protein Using Oct4 Promoter to Generate a Transgenic Mouse

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Abstract: Transgenic mice are extensively used to study stem cells and induced pluripotent stem cells (iPSCs). The aim of this study was to generate a transgenic mouse in order to study reprogramming of somatic cells into iPS cells. Methods: The sequence of oct4 gene promoter was obtained from GeneBank. By designing a set of specific primer, the promoter region was amplified and cloned into an engineered pEGFP-C1 vector containing three chS4 insulators. The expression cassette, including 2×chS4-Poct4-EGFP-TT-chS4, was excised by restriction enzyme digestion and injected into pronulci of 0.5 day mouse embryos. Results: Promoter of oct4 gene was amplified as a 2200bp fragment and cloned into an engineered vector. The presence of chS4 insulators in the vector reduces the chromosomal position effect on the target transgene. Injected embryos were transferred into the oviduct of a pseudo-pregnant mouse (foster). The founder mice will be identified after PCR analysis. Conclusion: The goal of this project is to generate a transgenic mouse that expresses EGFP gene under the control of oct4 promoter. One of the important properties of iPS cells is activation of oct4 promoter. Therefore, the somatic cells of such mouse will be able to express EGFP protein in response to reprogramming to iPS cells. This happen will eliminate numerous complex analyses to identify iPS cells.

Keywords: Stem Cell, IPS, Transgenic Mouse, Oct4
Title:
Signal amplification of a chemiluminescent aptamer based nanobiosensor for detection of Retinol Binding Protein 4 (RBP4)

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Abstract:
Introduction: Aptamers are small single stranded oligonucleotide with well defined three dimensional structures that bind to various biomolecules with high affinity and specificity. The biosensors in which aptamers are applied as biocomponents are called as aptasensors that are simple and able to detect biomarkers with high sensitivity. Retinol binding protein 4 (RBP4) is a useful biomarker in the prediagnosis of type 2 diabetes.

Methods: In this study, a chemiluminescent (CL) aptamer biosensor was fabricated by using polyclonal anti RBP4 antibody as capture probe and immobilizing luminol labeled aptamer on the surface of gold nanoparticle as reporter probe. Gold nanoparticles are effective in signal amplification for biosensing.

Results: The change of CL intensity reflected the concentration of RBP4. We observed wider linear range of the signal with a lower detection limit.

Conclusion: The approach showed a high sensitivity for the detection of RBP4.

Keywords: Aptamer, Biosensor, Gold nanoparticle, RBP4
Title:
The role of signaling of H2O2 in resistance of suspension- cultured tea cells to aluminium

Authors:
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Abstract:

Aluminum (Al) toxicity is a major factor that limits plant growth and development in acid soils. However, some plants, known as Al accumulators among which tea plants are typical ones. It has been shown that Al increases activities of antioxidant enzymes and growth of tea plants. In the present research, suspension-cultured tea cells were treated with 400 µM Al, for 6, 24, 48 and 96 h. The level of damage of membranes was determined by measuring malonyldialdehyde (MDA) as the end product of peroxidation of membrane lipids. Viability of the cells were assessed with Evans Blue (aqueous, 0.05% w/v) and the content of hydrogen peroxide (H2O2) as a signaling molecule was evaluated. Although no change was observed in growth (fresh weight) and viability of the treated cells with Al for 6 and 24 h treatment, but these factors were increased for 48 and 96 h, in comparison with those of the control cells. Treatment for 6 and 24 h had no significant effect on membrane lipid peroxidation, but decreased it 48 and 96 h of the treatment. No change was either observed in H2O2 content of tea cells at 6 and 24 h, but changed after 48 and 96 h. These results suggested that Al treatment of tea cells decrease their H2O2 and peroxidation of membrane lipids in a time-dependent manner which resulted in increase of the growth and viability of the cells.

Keywords: Aluminium, Camellia sinensis, Hydrogen peroxide, Malonyldialdehyde (MDA)
Title: Fatty acid composition in differentiation of tonsillar disorders


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Abstract:

Introduction: Obstructive and inflammatory diseases of tonsils are considered as the most common disorders of childhood. Fatty acids and most of its biological activities have been attributed to its capacity to generate biologically active lipids, which may sustain inflammation. They have been proposed as evaluating markers for infection and inflammation in tonsils. The aim of this study was to investigate the tonsillar fatty acid composition in pediatric cases undergoing tonsillectomy.

Method: The study of population included 205 children who had undergone tonsillectomy. Tonsil tissue samples fixed and were examined by making and preparation of pathology slides, 114 had hyperplasia and 91 chronic tonsillitis. On the other hand, tonsillar lipids were extracted and fatty acids analyzed by gas liquid chromatography. At last, they were compared with pathological evaluation.

Results: Palmitoleic, oleic and monounsaturated fatty acids were associated with chronic tonsillitis and saturated fatty acid stearic acid were higher than in tonsillar hyperplasia (P < 0.01).

Conclusions: According to the results of this study, tonsillar fatty acids composition was various in two cases of recurrent tonsillitis and tonsillar hyperplasia. Then, they have an important biochemical role in tonsillar disorders and may be considered as supportive diagnostic markers to differentiation of two groups.

Keywords: Tonsillar disorders, Tonsillitis, Tonsillar hyperplasia, Fatty acids composition, Children
Title: Hierarchical clustering of bacteria based on Fourier-transform infrared spectroscopy and molecular data

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Abstract: Introduction: Up to now, several methods of classifying bacteria based on morphology, metabolism, pathogenicity, and molecular properties have been introduced. Recently, hierarchical clustering of bacteria based on biochemical profile and cell surface characteristics has been developed. Fourier-transform infrared spectroscopy (FTIR) is a rapid, culture-independent and economic physico-chemical method to analyze the biochemical characteristics of bacterial surfaces and used successfully as a fingerprinting technique for classification of bacteria such that is comparable with 16SrRNA classification. In this study to investigate the phenotype differences between halophilic bacteria and classification based on cell surface organic compounds, FTIR data is used. Also, hierarchical clustering according to FTIR information is compared with phylogenetic tree based on 16SrRNA.

Method: Four different halophilic bacteria were isolated and to molecular identification PCR reaction was performed. Also, hierarchical clustering according to partial sequence of 16SrRNA by CLC sequence viewer software was drawn. To identification of cell surface compounds of each bacterium FTIR spectroscopy analysis was performed. Hierarchical cluster analysis based on FTIR spectra using the Specwin 32 and SPSS software was carried out and compared with 16SrRNA phylogenetic tree.

Results: Sequence blast analysis against the 16s ribosomal RNA sequences (bacteria and Archaea) in the NCBI database showed 4 isolated bacteria are members of different taxonomic groups. Hierarchical clustering based on 16SrRNA and FTIR spectroscopy data indicate two clusters have approximately the same branching pattern.

Conclusion: FTIR spectroscopy is a powerful and easy technique to discrimination and classification of bacteria and is comparable with phylogeny of bacteria based on 16SrRNA.

Keywords: FTIR, Hierarchical clustering, bacteria
Title:
Trichostatin A Induced Mir-122 Expression in Adipose Tissue-Derived Mesenchymal Stem Cells

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Abstract:
Introduction: Adipose tissue-derived mesenchymal stem cells (AMSCs) are good candidates for autologous stem cell therapy. They can not only differentiate into mesodermal lineages but also, trans-differentiate into hepatocytes and other epithelial cells. Recent studies showed that microRNAs (miRNAs) are regulators of differentiation and cell fate. Mir-122 is a liver specific microRNA with functional roles. Histone deacetylase inhibitors such as trichostatine A (TSA) exhibited differentiation stimulating properties. In the present work, we investigated the effect of TSA on expression levels of mir-122 in AMSCs.

Methods: Subcutaneous adipose tissue was obtained with informed consent from 6 donors by surgeon in Imam Reza Hospital of Tabriz, Eastern Azerbaijan, Iran. AMSCs were isolated using digestion with collagenase type I and following stages. Flow cytometry were used for the staining of AMSC markers with mouse anti-human FITC and PE conjugated antibodies including: CD34, CD31, CD11b, CD90, CD44, CD73, CD105, and CD745. Multi-potency of AMSCs was evaluated by osteogenic and adipogenic differentiation. AMSCs were cultured in two media composed of L-DMEM, bFGF, EGF, and OMS with or without TSA, respectively. MTT and colony forming assays was performed. The expression of mir-122 was investigated by LNA-based Real time PCR in cultured AMSCs at days, 7th, 14th, and 21east. Results: The majority (96%) of AMSCs were CD44, CD73, and CD90 positive. Epithelial-like cells were observed surrounded by fibroblastic cells 21 days after culturing with TSA. Additionally, TSA induced expression of mir-122 in TSA treated AMSCs.

Conclusion: The findings of this work may be applicable in production of functional hepatocytes from AMSCs utilizing mir-122 and TSA.

Keywords: Trichostatin A, Mir-122, Expression, Adipose Tissue-Derived Mesenchymal Stem Cells
Title: 
The methanolic extract of Teucrium persicum is toxic to cancer cells

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Abstract: 
Introduction: *Teucrium persicum* is an endemic plant of Iran which its pharmacologic properties have not been previously investigated.

Methods: The methanolic extract was isolated from aerial parts of Teucrium persicum and was used for measuring its toxicity against several human cancer cell lines. Cell viability was examined by MTT assay.

Results: The results of this study show that the methanolic extract, isolated from the aerial parts of *Teucrium persicum* dramatically inhibits cell proliferation of PC-3 prostate cancer cells with the IC50 value of 150µg/ml. The cytotoxic effects of *Teucrium persicum* are comparable to those of known anticancer drugs such as Doxorubicin, Imatininb and Carboplatin. *Teucrium persicum* extract also inhibited proliferation of T47D (breast) and SW480 (colon) cancer cells. The extract at 50µg/ml and 150µg/ml concentration values decreased proliferation of T47D cells by 55% and 65% respectively and the same concentrations inhibited SW480 cell proliferation up to 80%.

Conclusion: In summary, our results indicate that *T.persicum* has potential anticancer properties. Studies are underway to identify the mechanisms of action.

Keywords: Teucrium persicum, methanolic extract, cancer cell cytotoxicity
Title: Development of Testicular Capsule and Rete Ducts in The Ostrich (Struthio camelus) Embryo

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Abstract: Introduction: Although there is growing knowledge about the testicular capsule and rete ducts in mammals, very little information in other vertebrates is available. The only developmental study on the rete testis of ratite birds including ostrich has carried out by Budras and Meier (1981). Methods: Fertile ostrich eggs were incubated for 20, 26, 36 and 42 days then testicles of male embryos were separated and their 5-µm paraffin sections were stained by H&E, PAS, Masson’s trichrome and alcian blue. Images were captured by a light microscope equipped with digital camera. Results: 20-d-old embryo: There were some lacunar ducts in the attachment site of testis and kidney, while testicular capsule was limited to a covering epithelium and its underlying blood vessels. 26-d-old embryo: A loose connective tissue (tunica albuginea) appeared in the capsule while some organized cell aggregations were trapped inside it. These aggregations situated in the larger cisterns and in some areas they were transforming to tubule or duct-like structures. 36-d-old embryo: Tunica albuginea approximately changed to a dense connective tissue and majority of duct-like structures transformed to the capsular rete ducts. 42-d-old embryo: Tunica albuginea became completely dense and its meandering rays, defective septa, penetrated the interstitial tissue while holding some blood vessels. Conclusion: Tunica albuginea emerges in later embryonic stages while intracapsular rete ducts coordinately develop inside it. Capsular rete ducts in newly hatched chickens like the juvenile ostrich are spread along the capsule from orchido-epididymal border to the free surface of testis and their probable function is participating in semen transit.

Keywords: Embryonic development, Rete testis, Testicular capsule, Seminiferous tubule, Ostrich
Title:
Molecular Characterization of Transcriptional Regulatory Sequences of Pseudomonas fluorescens FY32 1-Aminocyclopropane-1-Carboxylic Acid Deaminase Gene

Authors:
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Abstract:
Some of plant growth promoting bacteria contain the enzyme ACC deaminase that catalyzes degradation of 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene, into α-ketobutyrate and ammonia. ACC deaminase gene, acdS has a unique promoter and regulatory system. The acdS regulatory system consist of several binding site and acdR. The AcdR is a regulatory protein that positively regulate acdS. Most of bacterial regulatory system are general and regulate expression of several gene and operon in same time then a unique promoter and regulatory system is very usefull for genetic engenering. Because of we can increase experression of a gene without effect on others. The acdS has different expression in different specie of bacteria then acdS must have different regulatory system in this specie.

Previous study suggesting horizontal transfer of the acdS gene and considering acdS sequences submitted in NCBI we can classify three homologues family of acdS in bacteria. In this study we identifying and sequencing the acdS promotor and regulatory system in Pseudomonas Fluorescens FY32. We designed primers to detection and amplifying acdS by PCR. Then PCR product, a 1727 bp fragment, was cloned in plasmid and transferet to E.coli DH5α. The ACC deaminase expression was detected and measured by α-ketobutyrate assey. Finally the extracted acdS was sequenced and compared with other submitted sequence in NCBI.

Keywords: acdS , acdR , ACC deaminase , Regulatory system , Pseudomonas Fluorescens FY32
Title: The effects of glibenclamide on the kinetic parameters of esterase activity of human carbonic anhydrase II

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Abstract: Carbonic anhydrases (CAs, EC 4.2.1.1) are widespread zinc containing metalloenzymes found in animals, plants, and bacteria. The enzyme catalyzes the reversible inter-conversion of CO$_2$ and HCO$_3^-$. This reaction is the main role of CA enzymes in physiological conditions. Most of the CAs can be inhibited by sulfonamides and their derivatives which are specific and strong inhibitors of CAs.

In this study, we used UV-Vis spectroscopy to investigate the effects of glibenclamide, a sulphonylurea drug, on the kinetic parameters of human carbonic anhydrase II (hCA II) esterase activity. The results showed that glibenclamide is able to inhibit carbonic anhydrase activity in the range of 10-400 µM with the IC$_{50}$ of 82 µM. Analysis of the Lineweaver–Burk plot revealed that glibenclamide has no effect on $V_{max}$ but increases $K_m$ for the esterase activity of hCA II and inhibits it via a competitive manner. By plotting $K_m$ against different concentrations of the drug (secondary plot), the $K_i$ was determined as $22.90\pm2.64$ µM.

Keywords: Human carbonic anhydrase II, Glibenclamide, Kinetic study
Title: Evaluation of Antioxidant and Wound Healing Effects of Aqueous Extract of Elaeagnus Angustifolia Fruit in Rats

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Abstract: Introduction: Wound healing is an important physiological event after birth and proper healing of wounds is essential for restoration of skin integrity and function. The present study was carried out to investigate the effect of Elaeagnus Angustifolia fruit extract on experimental wounds and lipid peroxidation levels in rats. Methods: After creating full-thickness skin wounds on the back of 45 male Sprague-Dawley rats, they were randomly divided into three groups including treated group which received the extract, positive control group treated with mupirocin ointment 2% and control group which did not received any treatment. Wound healing rates were calculated on days 3, 5, 8, 10, 12 and 15 post-wounding and the wound tissues were harvested at 5, 10 and 15 days for biochemical and histological analysis. Malondialdehyde (MDA) level, as a marker of lipid peroxidation, was measured in the wound tissue of rats. Results: The percentage of wound contraction was significantly higher at 10, 12 and 15 days in treated group compared to control but at 12 and 15 days than positive control. Histological scores were significantly higher at 10 and 15 days in treated and positive control than control. A significant decrease was also observed in MDA content of the skin of treated group in comparison to control and positive control groups. Conclusion: These data provide evidence that Elaeagnus Angustifolia extract has antioxidant properties through possessing the active compounds such as flavonoids (polyphenols), terpens and sitosterols, which may be responsible for faster wound healing. Therefore this extract can be used as a therapeutic agent for wound healing.

Keywords: Wound Healing, Antioxidant, Elaeagnus Angustifolia, Histology, Rat
Title:
Spectrophotometric Quantitation of Mebeverine in Bulk Drug and Pharmaceutical Formulations using Multivariate Calibration Technique

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Abstract:

Introduction: Mebeverine hydrochloride is 4-[ethyl(4-methoxy-α-methylphenethyl) amino]butyveratrute hydrochloride having molecular formula C_{25}H_{35}NO_{5}HCl, molecular weight 466 and melting point 105 -107 ºC. It is white or almost white, crystalline powder, freely soluble in water and ethanol (96 %), while practically insoluble in diethyl ether.

Method: A sensitive and accurate UV spectrophotometric method with multivariate calibration technique for the determination of mebeverine hydrochloride in bulk drug and different pharmaceutical formulations has been described. This technique is based on the use of the linear regression equations by using relationship between concentration and absorbance at five different wavelengths.

Results: The results were treated statistically and were found highly accurate, precise and reproducible. The method is accurate, precise and linear within the range 5-80 µg/ml (r=0.9966).

Conclusion: Under optimized conditions the applied numerical method provides considerable resolving power, sensitivity, rapidity, and low cost for the quantitative analysis, quality control and routine analysis of subject compounds. There was no interference from the excipients i.e Povidone K 30, magnesium stearate, lactose and hydroxypropylmethylcellulose. This statistical approach gives optimum results for the eliminating fluctuations coming from instrumental or experimental conditions.

Keywords: Mebeverine, Multivariate, Pharmaceuticals
Title:
Determination of Glycated Hemoglobin using boronic acid-coated CdTe Quantum Dots based on Fluorescence Resonance Energy Transfer

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Abstract:
Introduction: Glycated hemoglobin (HbA\textsubscript{1c}) is formed by a nonenzymatic and approximately irreversible reaction of glucose with the N-terminal valine of adult hemoglobin’s β -chain (HbA\textsubscript{0}). Since the lifetime of hemoglobin in blood is approximately 2–3 months so measuring HbA\textsubscript{1c} is well known for monitoring long-term glycemic control clinically. International Federation of Clinical Chemistry (IFCC) units (mmol/mol) and National Glycohemoglobin Standardization Program (NGSP) units (%) per total hemoglobin are two major standard for reporting HbA\textsubscript{1c}. The methods for measuring of HbA\textsubscript{1c} are based on charge differences (such as ion-exchange high-performance liquid chromatography) and structural differences (such as Boronate affinity chromatography and immunoassay) between the glycated and non-glycated species.

Method: Based on cis-diol binding ability of boronic acid, we coated CdTe quantum dots with 3-aminophenylboronic acid to bind with sugar moiety of HbA\textsubscript{1c}. Because of high extinction coefficient of Porphyrin, Hemoglobin act as a good acceptor in Fluorescence Resonance Energy Transfer (FRET) and we studied this mechanism between quantum dots and Porphyrin of hemoglobin.

Results: We found that fluorescence of quantum dots decrease linearly with increasing percentile concentration from 3% to 16% HbA\textsubscript{1c} per total hemoglobin.

Conclusions: This method measures total glycated hemoglobin, including HbA\textsubscript{1c} and other glycated hemoglobin derivatives, and has potential of developing to determine the concentrations of a variety of glycoproteins that contain peripheral sugar moieties.

Keywords: glycated hemoglobin (HbA1c), Quantum Dots, Boronic acid, Fluorescence Resonance Energy Transfer (FRET)
Title:
Investigating the Role of EF-hand I on the Function of Photoprotein Mnemiopsin From Ctenophore Mnemiopsis Leidyi Using Site-directed Mutagenesis

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Abstract:
Introduction: Light-emitting proteins are integral to a wide variety of scientific techniques, from the development of miniaturized biosensors to the non-invasive imaging of cellular and subcellular events and mechanics. All Ca^{2+}-regulated photoproteins show high sequence homology in loops and amino acid sequences. They contain three EF-hand Ca^{2+}-binding centers as characteristic of the family of Ca^{2+}-binding proteins.

Methods: A mutation of E50G in mnemiopsin was obtained using site-directed mutagenesis. During the following steps some important characteristics of the natural and mutant photoprotein such as, optimum pH and temperature, calcium sensitivity as well as incubation time were investigated.

Results: According to the results, both native and mutant photoproteins were extremely sensitive to pH, so that their activity at pH 9.0 was completely different with other pH values. It was also shown that calcium sensitivity in both native and mutant forms of mnemiopsin were lower than aquarin.

Conclusion: Sequence analysis together with the results obtained from the Motif Scan revealed that the native mnemiopsin has lost its first EF-hand. Therefore, although calcium sensitivity in mutant form of E50G had been increased compared to aquarin, it still show low sensitivity.

Keywords: Bioluminescence, Photoprotein, Mnemiopsin, site-directed mutagenesis, EF-hand.
Title:
Evaluation of blood urea and creatinine levels in pregnant women with different ABO blood groups

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Abstract:
Introduction: The term “blood group” refers to the antigen phenotype, results from the expression of the inherited genes, including A, B, and O in the ABO system. Based on previous findings in different studies the incidences of some diseases such as diabetes, coronary heart disease and some type of cancers are associated to blood types. Serum urea and creatinine levels is a well-known clinical demonstration of renal and kidney function and predictor of hypertensive disorders during pregnancy. In this study we investigated the association between serum urea, creatinine and ABO blood types.

Materials and methods: The blood samples were collected from 800 healthy pregnant women (including 4 type of blood groups with Rh+ in an equally manner) in the second trimester of pregnancy. Measuring serum urea and creatinine levels and blood typing test were performed by standard methods and then data was analyzed by spss16.

Results: The urea level in AB blood group was higher than the three other groups and it was higher in B compared to O and A blood group (p < 0.05); also the creatinine level in AB blood group was higher than the three other groups and it was significantly higher in B compared to A blood group (p < 0.05).

Conclusion: Our findings indicated that ABO blood group had the association with some of the risk factors of an unfavorable outcome of pregnancy and it may be one of the prognostic tools, also it addresses more extensive studies.

Keywords: Urea, creatinine, blood group, pregnant women
Title:
Investigation of tautomerism AcrylAmide on the fullerene (C30) in gas/solvent phase with the use density function theory calculations.

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Abstract:
On the theory of computing today, as the Nano Fullerene enjoy quite a lot of importance on. In this research with the push AcrylAmide (AcA) on the Nano Fullerene (C30), was studied balance tautomeric of 1,3 hydrogen atom transfer between (O and N) Therefore, in order to optimize energy and quantum computing using structures in the gas phase and with the water and methanol molecules assistant and in the solvent phase (water, methanol) with method Density Function Theory (DFT) in B3LYP level and 4-31G basis set (298.15K and 1atm) is achieved. Using the same frequency on level computing was studied thermodynamic functions such as ΔH, ΔG, ΔS and Keq. The results show that In all state the more stable form of the keto form is enol. the best state, which has the lowest is barrier energy activation in the presence of one molecule of water.

Keywords: Nano fullerene, AcrylAmide, Tautomeric, Thermodynamic functions
Title:
Association of allelic frequency of single nucleotide polymorphism -1099A>G in the promoter region of miR-33b with total HDL levels in the children affected with metabolic syndrome

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Abstract:

Background: Despite advances in the prevention and management of metabolic syndrome (MetS), this multifactorial disorder has been considered as one of the leading cause of mortality throughout the world. MetS is a prevalent disease in children and youths and has been considered as a major risk factor for diabetes mellitus and atherosclerotic cardiovascular disease. Insufficient or excessive cellular cholesterol results in pathologic processes including atherosclerosis. MiR-33 was identified as a key post-transcriptional regulator of cellular cholesterol homeostasis which is located in intron 17 of the SREBP-1 gene. In this study, for the first time, the association of -1099A>G polymorphism in the promoter region of miR-33b with the total HDL levels and the risk of developing metabolic syndrome in youths has been investigated.

Methods: In this case-control study on children and adolescent with MetS, all subjects were chosen according to the ATPIII criteria. A total of 100 patients and 100 controls were selected for this study. DNA extraction from peripheral blood was performed using Diatome kit. Allelic frequencies were determined by TaqMan Real-Time PCR.

Results: In patients, 21% were AA, 43% were AG and 36% were GG while in healthy individuals 27% were AA, 45% were AG and 28% were GG. Furthermore, the HDL levels in patients and healthy individuals were 43.6±5.3 mmol/l and 49.5±12 mmol/l, respectively

Conclusion: Statistical analysis (ANOVA) showed that levels of HDL in MetS patients with GG genotype was significantly higher than those in patients with AA genotype (P=0.03).

Keywords: Metabolic Syndrome, miR-33b, TaqMan Real-Time PCR
β-Casein nanoparticles as an efficient drug carrier system for curcumin and its derivatives

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Abstract:
In milk β-casein micelle exists a natural nanostructure, which can be exploited as a nano carrier of hydrophobic drugs. curcumin, diacetyl curcumin and bis-demethoxy curcumin, are the active components of turmeric(Curcuma longa L.) with many physiological, biochemical, and pharmacological properties. here, we investigated the complex formation of curcumin and some of derivatives with β-casein micelle and its use as a vehicle for drug delivery to cancer cells. Steady-state fluorescence spectroscopy of the β-casein micelle-curcuminoid complex formation revealed that at pH 7, curcuminoid molecules bind to β-casein micelle and formed complexes through hydrophobic interactions. the binding parameters including number of substantive binding sites and the binding constants have been evaluated by fluorescence quenching method. results showed that curcuminoid molecules quench the intrinsic fluorescence of β-casein upon binding. the distance(r) between donor (β-casein) and acceptor(curcuminoid) was obtained according to förster theory of non-radiative energy transfer. we evaluated the utility of β-casein micelle as carriers of curcuminoids by using in vitro cultured MCF7 cells. cytotoxicity studies of MCF7 cells revealed the higher cytotoxic effects of encapsulated curcuminoid on MCF7 cells compared to equal dose of free curcuminoid. also, a molecular docking study has been done on system for clearer visualizing of binding sites. molecular docking calculations suggested that curcuminoid bind to the hydrophobic core of β-casein micelle.

Keywords: Curcumin , Diacetylcurcumin ,Bis-demethoxy curcumin, β-Casein micelle, Fluorescence, Förster’s theory
Title:
Enzymatic kinetic in imidazolium based ionic liquid

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Abstract:

Introduction: ionic liquids (ILs) provide a new generation of solvents entirely composed of ions and are usually considered as green solvents. Biotechnological applications of ILs are currently increasing. This study aims to investigate the mechanism by which an ionic liquid may enhance the rate of biocatalysis.

Method: Enzymatic activity of horse liver alcohol dehydrogenase was measured by following the reduction of NAD$^+$ in different concentration of 1-butyl-3-methylimidazoliumbis(trifluoromethylsulfonyl) imide; [BMIM][NTf2]. The kinetic parameters of the enzyme ($k_m$, $V_{max}$ and $k_{cat}$) were obtained by UV-visible spectroscopy using michaelis menten equation. Structural assessment were performed to find the structure-function relationship.

Result: The obtained results showed that [BMIM][NTf2] led to reduction of $K_m$ and increasing the enzyme performance.

Conclusion: Alcohol dehydrogenase from Horse liver remain active in [BMIM][NTf2]. Moreover, structural analysis showed that the used IL brings about alteration in the secondary structure of the enzyme. The obtained results would introduce [BMIM][NTf2] as a good alternative for normal organic solvents.

Keywords: ionic liquid, enzymatic kinetic, alcohol dehydrogenase
Title:
Construction of an expression vector containing Melanoma differentiation-associated gene-7 (MDA-7) as a tumor suppressor

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Abstract:
Background and Aims: Melanoma differentiation-associated gene-7 (MDA-7)/interleukin-24 (IL-24) is a unique tumor suppressor gene, which has suppressor activity in a broad spectrum of human cancer cells. In this study, an expressing vector expressing Mda-7 was constructed and then evaluated for its integrity and protein expression.
Methods: Melanoma differentiation-associated -7 (MDA-7)/interleukin-24 (IL-24) gene was amplified by PCR. After purification and cloning the gene into TA-cloning vector, it was evaluated by restriction digestion analysis. The resultant Mda-7 was sub-cloned into constitutive expression vector then evaluated by sequencing and restriction digestion analysis. It then was transfected into 293 cells and its expression was evaluated by RT-PCR, fluorescent microscopy and Immunofluorescence assay.
Results and conclusion: Amplification results of mda-7 gene by PCR showed expected band size in gel. Vector evaluation by different methods confirmed the integrity of construct rather than inside gene. Suitable Expression of Mda-7 protein and its mRNA in 293 cell confirmed by RT-PCR, GFP detection and Immunofluorescent assays. Immunofluorescent assay showed suitable expression of Mda-7 in those cells expressing GFP. This construct must be evaluated for anti tumor effect in vitro.

Keywords: Mda-7, apoptosis, tumor
Title:
Dickkopf related protein -1 and bone mineral density levels in osteoporosis women

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Abstract:

Introduction: Osteoporosis is a common disease characterized by low bone mass that results in fragility fractures. In bone the Wnt signaling pathway has diverse roles in bone modeling and remodeling. Dickkopf related protein -1 (Dkk1) is endogenous inhibitors of the canonical Wnt/β-catenin pathway specific to bone.

Methods: The study population includes 44 women with osteoporosis and 44 controls with normal bone mineral density (BMD). BMD of Hip with femoral neck, and lumbar spines over L2–L4 regions were measured with Dual-Energy X-ray Absorptiometry and expressed as g/cm² using. Serum levels of DKK-1 and were measured by commercially available ELISA kits.

Results: The serum Dkk1 concentration in the osteoporosis group (2.91±1.27) was significantly increased compared to the control group (2.01±.87) (p<0.01). Osteoporosis patients had increased values of L1-L4 BMD (.71±.10 vs .98 p<0.001), (Hip) FN-BMD (.62±.10 vs .89 p<0.001) compared with controls.

Conclusion: In conclusion, the results of this study indicate that there are significant increases in serum levels of DKK1 compared with the control group. Also we found that Dkk1 was highly correlated with FN-BMD.

Keywords: osteoporosis, Dickkopf related protein -1, bone mineral density
Title:
Finding Micro-RNAs involved in VEGF signaling pathway by using bioinformatics methods and gene- microRNA interaction network

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Abstract:
The growth of new blood vessels (angiogenesis) in adults is occurred by budding of vascular endothelial cells, under certain physiological conditions such as wound healing and the female sexual cycle. Angiogenesis plays a key role in growth of tumor and metastasis and it was first proposed as a possible target for the treatment of cancer several decades ago. During recent years, the discovery of VEGF - as the main mediator of tumor angiogenesis- have encouraged researchers to study the molecular basis of this process and find ways in order to inhibit it. Mutations in the oncogenes and the tumor suppressors increase the concentration of angiogenesis stimulators -vascular endothelial growth factors- that they act through interaction with tyrosine kinases on the plasma membrane of the endothelial cells and set up a cascade of events within the cell. One of the main ways for regulation of genetic processes is related to the micro-RNAs. It is estimated that these molecules regulate the expression of one third of all genes. The purpose of this study is finding the most effective micro-RNAs for inhibiting the angiogenesis process. To this end, based on review of literatures and using the KEGG database, 28 genes was identified that involved in the VEGF signaling pathway. In the next step by using three different algorithms (DIANA, miRWalk, miRanda), and use of information contained in a databank of experimental studies (mirTarBase), the binding positions of micro-RNA on the 28 genes in this pathway were identified and some banks for this purpose were created, then by using softwares for studying biological networks, such as Cytoscape, 4 networks were created. The statistical studies conducted on 461 micro-RNA determined that 11 of them are present in 4 networks and the 77 micro-RNAs were at least in three of all networks. Finally those microRNAs that had more score -bind to most of genes or bind to more different positions on a single gene- were selected. The selected microRNAs can be good candidates for further investigation in culture cell and inhibition of VEGF signaling pathway.

Keywords: VEGF, microRNA, Bioinformatics, angiogenesis.
Title: The Influences of Different Concentrations of Benzyladenine on Proliferation of Ishtara Rootstock in In Vitro Situation

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Affiliation: Iran - tehran - hesark

Abstract: In this investigation, the propagation capacity of Ishtara rootstock which is a clonal peach rootstock hybrid was studied by proliferation. Apical and axillary buds of newly grown plant stem were used as explant material. explants are washed with water and treatment with hypochlorite sodium. Finally explants are rinsed three times with sterile distilled water. MS medium containing different concentrations of benzyladenine 0.0, 0.5, 1, 2 mg/l, 30 g/l of sucrose and 7 g/l Agar with pH 5.7 was tested. The cultures were incubated in culture room at 25 ±1 ºC. This experiment was conducted in Randomized Complete Design with 5 replications and All statistical analysis was performed using SAS software. Explants Shoots cultivated on the MS medium with 0.5 mg/l of BA produced the highest number of axillary shoots also 1 mg/l of BA concentration caused the highest number of adventitious shoots . In this concentration the use of BA at 0.5 mg/l level showed the best result for proliferation.

Keywords: Proliferation, Ishtara, MS medium, BA, rootstock.
Title: Amplifying GC rich regions in the promoter of Bcl2 gene

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Abstract: Introduction: The anti-apoptotic protein Bcl2 can protect cells against apoptosis induced by different agents and can increase resistance to anticancer drugs. Bioinformatic studies of Bcl2 gene, show a polymorphism in the promoter region of this gene. About 70% of human promoters have a high GC content and the promoter of Bcl2 is not exceptional from this fact. To amplify the promoter region of Bcl2 and investigate this polymorphism, due to high GC content and the formation of secondary structures, PCR setup will be time consuming and amplification of the regions of interest fail. The goal of this study is to provide optimal conditions to amplify GC rich regions in the promoter of Bcl2 gene.

Methods: In order to amplify the region of interest in Bcl2 promoter, in addition to the basic components of PCR reaction, two additional substances; Betain 5M and Dimethyl sulfoxide (DMSO) 5M and a high concentration of Mgcl2 were added to the PCR reaction. The combination of these factors was more effective in improving PCR products.

Results: Both additives greatly improved target product specificity and yield during PCR amplification.

Conclusion: There are instances in which standard PCR amplification conditions do not produce acceptable results. In these cases there are a number of additives that can be used to increase yield and specificity of a reaction. DMSO and Betaine are highly compatible with all other reaction components of gene synthesis and do not require any additional protocol modifications.

Keywords: Promoter, amplify, Bcl2, polymorphism, GC rich
Title:
effect of gamma iradiation on the somaclonal variation of artemisia aucheri boiss by using ISSR markers

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Abstract:
*Artemisia* is a medicinal plant belong to Asteracea family Anthemidea Tribe. it covers about 3/4 of iran plateau vegetation area. Due to richness of important components of medicinal materials this plant has come to consideration significantly . in this study *in vitro* culture with at least tree replications were used. *Artemisia* plants were cultured on MS medium and after one week plants were exposed to gamma radiation at 50-100-200 (Gr) after four weeks samples collected for ISSR-PCR . the genomic DNA was isolated from the radiated plants and control samples by using CTAB method and optimization of condition for molecular analytic. five primers were selected and the third primer (AC)8TA showed better results than other primers and identify variation . it seems that gamma radiation may cause somaclonal variation in this plant.

Keywords: gamma radiation - artemisia aucheri Boiss - ISSR-PCR
Title:
Identification and determination of synthetic pharmaceuticals as adulterants in eight common Herbal slimming supplements in Iranian market

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Abstract:
Objective: Adulterated herbal slimming products with undeclared synthetic drugs are common and responsible for many cases of serious health damage and occasionally even to death. The purpose of the study was to determine five synthetic adulterants in eight common herbal weight loss supplements sold in the Iranian market to verify that these substances are not mentioned on the labels.

Materials and methods: Eight common herbal slimming samples were obtained from Iranian market after advertising in satellite channels in Persian language and internet. Five pharmacological classes drugs used for weight loss, namely sibutramine, phenolphthalein, phenytoin, bumetanid and rimonabant, were investigated and quantified in them by GC-MS for the first three and LC-MS for the last second.

Results: The most undeclared ingredients which is added illegally were sibutramine, phenolphthalein, bumetanid, and phenytoin in Original super slim, Herbaceous essence, Green lean super slim, and Herbaceous essence respectively. Rimonabant was not found in them. Caffeine, pseudoephedrine, theobromine and amfepramone were also qualitatively found in the supplements by GC-MS library.

Conclusion: The life-threatening of illegally sold herbal weight-loss and dietary supplements have increased. These adulterated products have the health problem risks.

Keywords: adulterant, herbal slimming, weight loss, synthetic
Title:
Biodesulfurization of Dibenzothiophene to 2-hydroxybiphenyl by a newly isolated bacterium

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Abstract:

Introduction: The combustion of Hydrodesulfurization-refractory organic sulfur compounds such as Dibenzothiophene (DBT) in fossil fuels emits sulfur oxides, which can cause air pollution and acidrain. several types of bacteria was able to remove selectively the sulfur from DBT without breaking the carbon ring via 4S pathway; thus Biodesulfurization is a potential alternative method that can operate under mild conditions and does not decrease the value of the fuel.

Method: A new bacterial strain capable of selectively desulfurizing dibenzothiophene was isolated from oil contaminated soils collected in Khuzestan. Reduction of DBT was assayed by UV spectrophotometric analysis at the absorption maximum for DBT at 323nm. The desulfurized product of DBT, 2-hydroxybiphenyl (2-HBP), was identified by Gibbs assay and confirmed by HPLC.

Results: This isolate did not utilize DBT as the sole source of carbon or carbon/sulfur source. This bacterium was reduced about 90% of starting 0.3mM DBT within a 72-h after culture. 

Conclusions: Gram-positive, non-spore-forming, bacilli aerobic bacteria is oxidase negative and catalase positive showed high and stable ability for desulfurization of DBT. HPLC analysis and Gibbs assay were shown that DBT desulfurized via 4S-pathway. The results confirmed that this isolated bacterium could be used for the biodesulfurization of petroleum.

Keywords: Biodesulfurization, Dibenzothiophene, 2-hydroxybiphenyl, Isolation
Title:
Investigation of the interaction of anti asthma drugs with Human serum albumin (HSA)

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Abstract:
Human serum albumin (HSA) is the most prominent protein in plasma and has exceptional ligand binding capacity. Almost all pharmaceuticals released into the blood finds themselves in the presence of a high concentration of HSA, which is known to have strong affinity for various chemical compounds. Formoterol (FRM) and salbutamol (SAL) are potent b2-adrenergic receptor agonists widely used in the treatment of asthma disease. Here, Interaction of FRM and SAL with HSA was investigated by using molecular modeling, circular dichroism (CD) and isothermal titration calorimetry (ITC) measurements. The REDUCE software was utilized to add missing hydrogens to the X-ray crystal structure of HSA, and Autodock VINA was used to perform docking simulations. Different clefts in HSA structure were searched for potential high-affinity binding sites. The results were compared with known drug-HSA complexes.

Experimental measurements show that interaction of FRM and SAL with HSA does not induce considerable structural changes in protein. Docking results show that, in order of increasing affinity, FRM binds to drug site 1, FA site 6 and HEM binding site of HSA. The same analysis for SAL indicates that the highest affinity corresponds to HEM binding site followed by FA site 6 and drug site 1. Results of current study make a better understanding of the extent of distribution and side effects of anti asthma drugs in the blood and the interaction with the Human serum albumin (HSA) as a model protein.

Keywords: Keywords: Human serum albumin, formoterol, salbutamol, molecular docking, ligand-protein interaction
Title:
Retinoic Acid Receptor Overexpression in Human Umbilical Cord-derived Mesenchymal Stem Cells

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Abstract:
Introduction: Retinoic acid (RA) involves invertebrate morphogenesis, growth and apoptosis through two classes of receptors encoded by six genes; RAR(a, b, g) and RXR(a, b, g). The former utilizes either all-trans RA or 9-cis-RA as ligands, whereas the RXRs utilize only 9-cis-RA. Using the human umbilical cord derived stem cells (HUCSCs) as an in vitro model of human fetal cells we aimed to evaluate RAR overexpression following to RA treatment.

Methods: Human umbilical matrix derived mesenchymal stem cells (HUCSCs) were cultured in DMEM + 10% FBS at a density of 1 × 10^3/well. Upon adhering, the medium was changed to DMEM containing RA for 4-6 days during which RA refreshed every 2 days. The cells cultured without RA were considered as a control group. Using a combination of flow cytometry, MTT colorimetric assay and conventional RT-PCR techniques, CD markers, cell viability and RAR expression profile of HUCSCs were measured, respectively.

Results: Flow cytometry analysis clearly indicated 5.4% of HUCSCs co-expressed CD34 and CD45, while 63.7% of cells expressed both CD44 and CD73. 36.5% of cells expressed CD90 compared to 0.05% for CD105. MTT assay also showed that about 60% of HUCSCs viability decreased at higher doses (10^-7–10^-5) of RA compared to control group. RT-PCR analysis also revealed that RAR a and b were upregulated in the RA-treated cells.

Conclusions: This study clearly shows that the HUCSCs express CD44, CD73 and CD90 and RA in a dose-dependent manner has cytotoxicity effect on HUCSCs that is mediated by RAR a and b.

Keywords: Human Umbilical Cord-derived mesenchymal Stem Cells, RAR, RXR, Retinoic acid, Cytotoxicity
Title:
Pattern Formation in Drying Colloidal Droplets Containing E. coli Bacteria

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Abstract:
Introduction: A consummate understanding of an evaporating colloidal droplets has important consequences in emerging technologies such as micro and nanoscale array fabrications, nanowires, injectprinting, paint technology, and protein crystallization. The formation of so-called “coffee rings”, in systems involving hydroxyapatite nanoparticles and polystyrene latex microspheres has been explained using the concept of contact line "pinning". Recently, the scientists investigated the pattern formation of the animate objects such as bacteria and arrays of the single cells. In this paper we have studied the pattern formation in drying colloidal droplets containing E. coli. We have investigated the effects of some physical parameters such as concentration, temperature, volume and also the surface on which we put our droplets in two different cases. The first case is the one that the bacteria are alive and the latter is the one that the bacteria are dead. Method: We have used a specific strain of E. coli (HCB 137), which has no flagella in its structure and thus could not swim in our experiment. We used LB liquid medium for culturing the bacteria. In order to kill the bacteria we added formaldehyde solution to its medium. We used the phase contrast microscopy to see them with camera. To analyze the pictures we used ImageJ and MATLAB. We also investigated the dynamics of drying by taking some movies with CCD. Results: We observed that effect of volume on the formation of the patterns is negligible. However, other physical parameters such as temperature, concentration or the surface have a significant role in the pattern formation. For the droplets on microscope slides; we saw that concentric rings formed after drying. The number of these concentric rings was different in all of the samples. However, we could not distinguish between the dead and alive bacteria because are data were so dispersed. In contrast, for the droplets on coverslips; we saw that almost all of the samples there was only one outer ring. We calculated the mean density of the bacteria inside the droplets and also the width of the outer ring. We could not see any difference between alive and dead bacteria within the error margin. Furthermore, we observed that by increasing the concentration the width of the outer ring increased, as we expected. We also observed that in the high concentrations, the patterns of the drying in the dead and alive samples are totally different from each other. Moreover, we saw that by increasing the temperature the pattern formation of the drying was changed considerably. Conclusions: Although these physical parameters changed the pattern formation of the drying, we could not distinguish the dead and alive E. coli from each other. It was the expected result. Because, our bacteria sample did not have flagella in its structure and thus could not swim. It is seemingly that there are some differences in the patterns of drying between the dead and alive due to the inactivity of the hydrogen pumps in the dead samples which are effective in the diffusion of the bacteria in the droplets. In addition, formaldehyde might change the adhesion binding of the dead bacteria to the surfaces. However, the amount of these differences is negligible. We would like to investigate the effect of the motility of the bacteria in the formation of the patterns for our future works.

Keywords: Coffee rings, pinning, Contact line, colloidal droplets, E. coli.
Title: Investigation of anti-inflammatory effects of the Zhumeria Majdae Resh & Wendelbo extract on mixed glial cells of rat

Authors: Parastoo Ramazani1, Farzaneh Sabouni1, Sajjad Mohammad Gholiha1, 2, Forough Sanjarian1

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Abstract: Inflammation plays a key role in some neurodegenerative diseases that can be treated by medicinal plants. Zhumeria majdae is a medicinal plant, which has been used extensively in the traditional medicine of Iran. This plant`s extract contains terpenes and flavonoids, which have analgesics, anti-oxidant, anti-bacterial and anti-inflammatory effects. Mixed glial cells are non-neuronal cells in the brain that contain microgelia, astrocytes and oligodendrocytes. They are responsible for the safety of the CNS. In neurological diseases, microgelia induce inflammation with the secretion of inflammatory factors such as pro-inflammatory cytokines and neurotoxins. Subsequently they have ability to control diseases. One of these molecules is nitric oxide (NO) that is a major factor in assessing the degree of inflammation. In this study, we investigated anti-inflammatory effects of the extract on reduction of the NO in mixed glial cells. Neonatal rat primary mixed glial cells were isolated from new born rat (1-2 years old) cultured in DMEM supplemented with 20% fetal bovine serum (FBS). In 14th day of the culture, cells were isolated with trypsin and cultured in 96-well plates, and then treated with LPS and different concentrations of the extract. The experiment carried out by using Griess reaction test to evaluating NO production in the cells. Different concentrations of extract was performed in three replications and analyzed by SPSS software. The results that analysed by SPSS were significant and revealed that the extract of Zhumeria has anti-inflammatory effects on the cells, and reduced the amount of NO compared with positive control (LPS).

Keywords: zhumeria, anti-inflammatory, NO, CNS
Title:
Investigation of anti-inflammatory effects of the Milk thistle (Silbium marianum L.) extract on mixed glial cells of rat

Authors:
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Abstract:
Inflammation plays a key role in some neurodegenerative diseases that can be treated by using medicinal plants. Milk thistle (Silbium marianum L.) is a medicinal plant, that its alcoholic extract contains flavonoids, such as silymarin which has antioxidant, anti-bacterial and anti-inflammatory effects. Mixed glial cells are non-neuronal cells in the brain that contain microglia, astrocytes and oligodendrocytes. They are responsible for the safety and supporting of the CNS. In neurological diseases, microglia induce inflammation with the secretion of inflammatory factors such as pro-inflammatory cytokines and neurotoxins. Subsequently they have ability to control diseases. One of these molecules is nitric oxide (NO) that plays an important role in inflammation and is a major factor in assessing the degree of inflammation. In this study, we investigated anti-inflammatory effects of the extract on reduction of the NO in mixed glial cells. Neonatal rat primary mixed glial cells were isolated from new born rat (1-2 years old) cultured in DMEM supplemented with 20% fetal bovine serum (FBS). In 14th day of the culture, cells were isolated with trypsin and cultured in 96-well plates, and then treated with LPS and different concentrations of extract. The experiment carried out by using Griess reaction test to evaluating NO production in the cells. Different concentrations of extract was performed in three replications and analyzed by SPSS software. The results that analysed by SPSS were significant and revealed that silymarin has anti-inflammatory effects on the cells, and reduced the amount of NO compared with positive control (LPS).

Keywords: Silbium marianum, anti-inflammatory, silymarin, CNS
Title:
Discrimination of probiotic Lactobacillus strains isolated from Iranian traditionally dairy products by Ribosomal DNA sequencing and repetitive sequenced-based PCR fingerprinting

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Abstract:
Introduction: The use of Lactobacillus as probiotics requires the application of accurate and reliable methods for detection and identification of bacteria at the strain level. The aim of this study is evaluation and detection of genetic diversity of lactobacilli species isolated from different sources in Iran based on REP-PCR. Method: Twenty strains were isolated from Iranian traditional yoghurt, cheese, and Tarkhineh. PCR-mediated amplification was carried out by degenerate primers. Sequencing of 16s rDNA was performed after purification of the PCR product. The rep-PCR fingerprinting by REPIR-I oligonucleotide primer was carried out for discrimination of isolates. Dice similarity was determined among the strains studied and used for grouping of the genotypes by UPGMA clustering methods and PCA analysis. Results: Isolates were deposited as novel stains of Lactobacillus casei, brevis, plantarum, and Entrococcus facium in GenBank. The 20 isolates produced different banding patterns, with 13 visualized PCR products in the range of 200 to 2500 bp. Clustering methods performed on molecular data by two different software (NTSYS and Darwin), produced similar results which were also supported by PCA ordination plot. The REP-PCR fingerprinting grouped all studied isolates into a few clusters as four main clusters were observed in dendograms. In all analysis, isolates of Lactobacillus casei, brevis, plantarum, and Entrococcus facium form four separate clusters. Conclusions: The REP-PCR profiles showed that 20 type isolates produced different banding patterns. Thus it has been proved that REP-PCR appears to be a very practical method and highly sensitive in discrimination of the lactobacillus species.

Keywords: probiotic, genetic relationship, REP-PCR fingerprinting, PCA, UPGMA
Title:
The investigation of the function of Aquaporin-5 (AQP-5) in human lung by QM/MM

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Abstract:
Aquaporins (AQPs) are proteins embedded in the cell membrane that regulate the flow of water. Human AQP5 facilitates the transport of water across plasma membranes and has been identified within cells of the lungs. AQP5 is expressed in vascular endothelium lung, visceral pleura lung, and a subset of pneumocystis. A quantum mechanical molecular mechanical (QM/MM) method is a technique used to get around this hurdle, processes involving the breaking of chemical bonds. QM treatment is heeds. These studies have defined the structure for AQP5 protein in alveolar epithelium in human lung. We simulated transmission of ion and water with different softwares in this research we used computational software such as Hyper chem and Gaussian with 6-31 G base set and computed thermochemical functions such as change atomics charge, minimum and maximum atomics charge and HF. We used Gaussian 98 to investigate stability, frequencies and Nuclear Magnetic Resonance (NMR) on six basis set on AB initio and Density functional theory Methods. The systems were optimized at first by HF and BLYP, B3LYP methods by STO-3G, 6-31G, and with the same basis sets and methods. Results given the data we see that the absolute difference between atomic charges in the band 1-100 value is 4.018562. The abundance of AQP5 on the apical surface of lung suggests a role in alveolar transmembrane water movement. These data indicate that these groups influence the properties of these molecules movement.

Keywords: AQP5, Gaussian, lung and atomic charge
Title:
Investigation on the immunoreactivity of β-lactoglobulin from milk of different species.

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Abstract:
Introduction: β-Lactoglobulin (β-Lg) is the major whey protein of ruminant species and is also present in the milks of many, but not all, other mammal species. Its biological role is still not well known, and many studies have suggested a nutritional and a specific transporter role. It is also one of the most allergen proteins in the milk of ruminant species. In the present work, it is evaluated the binding of IgE from cow’s milk allergy (CMA) patients to β-Lg form different species.

Material: A series of 25 sera from CMA patients presenting various symptoms were used. For each patients, the specific IgE titer was determined for goat and bovine β-Lg. The experiment was repeated three times. All the specific IgE binding results confirmed by IgE binding inhibition experiments.

Results: The binding of IgE from patients having CMA on β-Lg from goat’s and cow’s milk were studied by Fluorimetric ELISA techniques. The mean IgE binding to goat's β-Lg is higher than cow's β-Lg. Lower binding of β-Lg specific IgE to goat's β-Lg in comparison with bovine one was confirmed by IgE-binding inhibition experiments. Calculated IC50 was 0.21 and 0.89 µg/mL for goat's and cow's β-Lg respectively, indicating that goat's β-Lg is less recognized than bovine one by IgE from CMA patients.

Conclusion: It has been showed that β-Lg isolated from goat's milk has weaker binding of IgE from CMA patients compared with the bovine one.

Keywords: β-Lactoglobulin, Allergy, ELISA techniques, IgE binding
Title:
In silico comparison of synonymous codons usage between coding sequence of low and high risk Human papillomavirus genomes

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Abstract:

Introduction: Human papillomaviruses (HPVs) are small and nonenveloped viruses with double-stranded DNA. The genome of HPVs encodes a number of early and late proteins. These viruses can be classified in to high risk and low risk groups according to production of malignant lesions.

Method: In the present study, synonymous codon usage in coding sequence of high and low risk human papillomavirus genomes was analyzed. Synonymous codon usage of these two groups was obtained from database http://www.kazusa.or.jp/codon analyzed using of ROC software.

Results: Statistical data analysis indicated that usage of five codons of UAU(tyrosin), UGU(cysteine), ACA(threonine), AGG(arginine), GUC(valine) is significantly different between high and low types.

Conclusions: The result revealed that codon usage analysis is useful for classification of high and low risk human papillomavirus.

Keywords: Key word: human papillomavirus, synonymous codon usage, bioinformatic, high risk, low risk
Title:
Effect of exogenous D-ornithine on antioxidant enzymes, viability, and growth of tobacco cells under salinity stress

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Abstract:
Salinity is one of the major factors limiting plant development and crop productivity. Much of the plants injury by salinity is associated with induction of oxidative stress. Accumulation of reactive oxygen species, is overwhelmed by the activity of scavenging enzymes, e.g., peroxidase and catalase. Application of certain amino acid enantiomers has been recently introduced as a potent method to increase plant resistance against environmental stresses. In this study, the effects of D-ornithine in the tolerance of suspension-cultured tobacco cells against salinity were investigated. Six day old tobacco cells were treated with 50 mM of NaCl with or without 1 mM of D-ornithine. Treatment with NaCl adversely affected the growth and viability of tobacco cells so that fresh weight of the cells was lowered by 50% of the control, when the cells were treated with 50 mM of NaCl. Salinity induced the activity of antioxidant enzymes, compared to the control conditions. However, it was not sufficient to scavenge H2O2. Exogenous D ornithine not only had no adverse effects on cell viability and growth but also has beneficial effects on catalase and peroxidase activity, thereby reinforced resistance of tobacco cells against salinity stress. Therefore, D-ornithine can be introduced as a promising candidate to increase the resistance of tobacco plants against salinity and probably other similar stresses which are accompanied by oxidative burst.

Keywords: Antioxidant enzyme, Ornithine, Salinity, Tobacco, Viability
Title:
Effect of lead on the growth and metal accumulation by Origanum majorana L.

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Abstract:
Introduction: *Origanum majorana* is an aromatic plant belonging to the family Lamiaceae. Medicinally it is used to cure various human ailments. The plant is bitter, hot, stomachic, anthelmintic and useful in diseases of the heart and blood. Lead is one of heavy metal contaminant in the environment and toxic element for plants. In this study, the growth and tolerance of this plant against Pb toxicity was tested, also the amounts of Pb accumulation in the shoots was determined.

Method: The seeds of *O. majorana* were sown in the pots containing perlite and Hogland nutrient solution feeding for 30 days, then they treated with different concentrations of lead (0, 5, 10, 25, 50, 100 ppm). After 3 weeks treatment, the plants were harvested and dried at 70°C. Then the biomass was determined. Dried sample was ground, digested in concentrated HNO$_3$ and HCl. Lead content of the extract was determined with flame atomic absorption spectrometer.

Results: Dry weights of roots and shoots decreased with increase in Pb concentration significantly. With increasing lead concentration from 5 to 100 ppm in the medium, shoot and root Pb concentrations were increased from 505 to 1045 (µg.g$^{-1}$) and 3162 to 26773 (µg.g$^{-1}$), respectively.

Conclusions: *O. majorana* is a tolerant plant to Pb which able to accumulate high amounts of Pb in their tissues. Further work is needed to examine whether the Pb treatment could also affect the *O. majorana*'s secondary metabolites.

Keywords: Origanum majorana, Lead, Accumulation.
Title: The physiological response of Garlic (Allium sativum L.) to nickel sulphate

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Abstract: The effect of different concentrations of nickel sulphate (NiSO₄ . 6 H₂O) on some physiological parameters in garlic (Allium sativum L.) were studied. The plants were grown in greenhouse over a period 8 weeks in soil containing nickel varying between 0 and 100 mg/Kg soil. Growth parameters, dry weight, fresh weight and shoot length increased but root length decreased significantly (p ≤ 0.05). All biochemical parameters, such as total chlorophyll, chlorophyll a, and carotenoids, but chlorophyll b, and MDA increased significantly (p≤0.05) compared to control plants. Chlorophyll b content showed no significant difference. Increased MDA in root and bulb, but not in leaf of treated plants indicated increased lipid oxidation in cell membrane in these organs compared to control. It was concluded that nickel sulphate in 42 μM induced the growth of A. sativum. Implication of this physiological change is discussed.

Keywords: Nickel sulphate, Allium sativum, chlorophyll, physiological response.
Title:
Effects of an active derivative of Spiro Quinazoline on apoptosis of breast cancer cell lines MCF-7

Authors:
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Abstract:
Background Breast cancer is the most common form of cancer and the resistance of breast cancer cells to the available chemotherapeutics is a major obstacle to successful treatment. Quinazoline is the main six-membered heterocyclic ring system reported for their biological activities and it has been used especially as an anti-malarial agent and in cancer treatment. In this study, we used human breast cancer carcinoma cell lines MCF-7 as an experimental model system and examined the growth inhibitory effects of one active derivative of Spiro Quinazoline. Methods The cell lines were cultured in RPMI medium and treated with different concentration of active derivative for different time lengths. Inhibition of proliferation was measured by MTT assay. The morphological changes of cells were observed by fluorescence microscope after acridine orange staining and the apoptosis of cells was examined by DNA agarose gel electrophoresis analysis of DNA fragmentation. Results MTT assay indicated that this active derivative treatment decreased the viability of MCF-7 human breast cancer cells and inhibited proliferation and induced apoptosis of MCF-7 cells in a dose and time dependent manner. Cell cycle distribution determined using flow cytometry of propidium iodide stained nuclei and autophagy was detected by acridine orange staining. Conclusions This active derivative inhibits proliferation of breast cancer cells MCF-7 by inducing cell apoptosis and further studies are require to evaluate effects of that on the apoptotic and anti apoptotic genes and proteins with RT-PCR and western blot methods.

Keywords: MCF-7 cell line, Apoptosis, Quinazoline
Title:
Value Added Products from Activated Sludge Residue

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Abstract:
Today the increase in urban population world over with concomitant growth in wastewater treatment plants has caused production of large volumes of wastewater sludge, which utilization and disposal are of the most difficult and expensive. Sludge generated from municipal wastewater treatment plants are mainly primary sludge and activated sludge, in which the activated sludge comes from the secondary treatment. Among the products that can be produced from the sludge are biofuel (biodiesel, bioethanol), enzyme, and biosurfactant. Pretreatment of sludge can increase the production yield and speed up the degradation of organic matter. There are various pretreatment methods for this purpose. The goal of the present study was to find the most efficient pretreatment for activated sludge to produce ethanol. *Saccharomyces cerevisiae*, an ethanol producer strain, was grown on both pretreated and untreated sludge. In order to release the nutrients present in the sludge three different pretreatments, i.e. acidic, basic, and ultrasonic were applied. The ability of growth of *S. cerevisiae* on the pretreated and untreated sludge was compared using CFU method. The results showed that, among the applied pretreatments, ultrasonic may be the most efficient pretreatment to open up the structure of sludge biomass. According to the results of this study *S. cerevisiae* could growth on ultrasonic pretreated sludge. However the ability of this microorganism to produce ethanol must be identified. Additionally, in order to maximize nutrient releasing, the conditions of the pretreatment need to be optimized.

Keywords: Wastewater, Biofuels, Activated sludge, Value added products, Pretreatment
Title:
The study of allelopatric effects of ferulago angulata root extract

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Abstract:
The study of allelopatric effects of ferulago angulata root extract. Ameneh. Ravansalar1,* , Seyed Mehdi. Razavi2, Alireza Ghasmiyan3, Shahabodin Mirinejad4 1,2,3 Department of Biology, Faculty of Science, University of Mohaghegh Ardabili, Ardabil, Iran 4 Agricultural and natural resources research center of Kohgilooie-va-boyerahmad province, Yasoj, Iran a.ravansalar@gmail.com Introduction:: Ferulago angulata ( Apiacea ), is a perennial herb distributed from mediteranian to western Asia. In the present work we studied the allelopatric activity of the plant methanolic extracts on seed germination, shoot and root growth of Lettuce(Romaino-Siahoo) and pigweed( Amaranthus retroflexusl). Method: Dry roots of F. angulata were extracted with soxhlet apparanatus and using methanol solvent. Five concentrations (0/001, 0/01, 0/1, 1 and 10 ) of the extract were prepared with dissolring in distillated water. All the experiments were preformed in four repliections using strilled pteridishes. 5 ml of the each concentration was to pteridish. A control test was performed using distilled water, as well as. Results: Results showed that the concentrations of extract has signification effect(p < .05) on root and shoot growth. and methanol extract of Ferulago angulata significantly showed phytotoxic effects on lettuce and pigweed. At the concentrations higher than 0/001 mg/ml, the extract significantly reduced root and shoot growth of both Lettuce and pigweed seedlings. Discussion: It was concluded that methanol extract of Ferulago angulata roots exhibit strong allelopatric effect. The observed allelopatric might be attributed to coumarins in plant roots. So, it can be candidate as natured hercieded for control of weeds. KEYWORDS: Allelopatric, Ferulago angulata, Lettuce, Pigweed,

Keywords: Allelopatric, Ferulago angulata, lettuce, pigweed.
Title: Interaction of gemini surfactant with alpha-lactalbumin

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Abstract: alpha-lactalbumin (α-LA) is a small globular protein with stable and detectable molten globule state in special conditions. Effect of different surfactants on α-LA structure has been studied. In this study the effect of cationic Gemini (12-2-12, 2Br-) surfactant on the structure of bovine and camel α-LA was studied, using fluorescence and circular dichroism spectroscopy techniques. The experiments were carried out in tris buffer (pH 7.5) containing calcium chloride 2 mM. Gemini surfactant at low concentration induced partially folded conformation in both α-LA species. α-LA secondary structure is stable against denaturation and it remains native-like in the presence of gemini, just small increase in alpha helical content of α-LA observed when the molar ratio of surfactant/protein reaches ten. Intrinsic fluorescence increased with increasing concentration range of the gemini and the maximum of emission shifted to longer wavelengths. It means that tertiary structure decreased with increasing concentration range of the gemini and it is accompanied with Trp exposure to the solvent. Concentration of gemini in the midpoint of transition, was higher for camel α-La than that of bovine counterpart. Favorable electrostatic interactions between the two charged head groups and the negatively charged centers of protein and also hydrophobic interactions between hydrocarbon tails of gemini with hydrophobic side chains of α-LA, both may influence protein denaturation. Interaction with gemini surfactants like other ionic surfactants leads to substantial conformational change of α-LA which may stimulate their ability of self-association or aggregation.

Keywords: Gemini, α-lactalbumin, Protein interactions
Title: Isolation of a β-amyrin Synthase from Chicory (Cichorium intybus L.)

Authors: Zhila Hossein Panahai and Asad Maroufi

Affiliation: Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran.

Abstract: Triterpenoid saponins are secondary metabolites showing a remarkable structural variety, as well as notable biological activities. They are synthesized via the isoprenoid pathway by specific oxidosqualene cyclases (OSCs) to give primarily oleanane (β-amyrin), ursane (α-amyrin), lupeol or dammarene-type triterpenoid skeletons. β-amyrin is catalyzed by β-amyrin synthase, which is one of the most commonly occurring triterpenes in nature and is defined as a typical pentacyclic triterpene having an oleanane skeleton. The gene encoding β-amyrin synthase has been isolated and characterized from different plant including species belong to Asteraceae family, a very good source of triterpenoid saponins. Chicory (Cichorium intybus L.) is one of the most important medicinal plants from this family. In this investigation, a 700 bp cDNA encoding a β-amyrin synthase was cloned from chicory by reverse transcription-polymerase chain reaction using degenerate primer designed from the conserved sequences among the known OSCs. This is the first identification of β-amyrin synthase cDNA from chicory. Successful identification of a beta-amyrin synthase has future biotechnology applications.

Keywords: Triterpenoid saponins, β-amyrin, Asteraceae, Cichorium intybus
Title:
Effect of cadmium on the growth and metal accumulation by Satureja hortensis.

Authors:
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Abstract:
Introduction: *Satureja hortensis* has a natural source of compounds for treatment of ailments includes camps, muscle pains, diarrhea and infection diseases. cadmium is a toxic element for plants. In this study, the growth and tolerance of this plant against Cd toxicity was tested, also the amount of Cd accumulation in the shoots was determined.

Method: The seeds *S. hortensis* of were sown in the pots containing perlite and Hogland nutrient solution feeding for 30 days, then they treated with different concentrations of cadmium(0, 2.5, 5, 8, 12,15 ppm). After 3 weeks treatment, the plants were harvested and dried at 70°C. Then the biomass was determined. Dried sample was ground, digested in concentrated HNO₃ and HCl. cadmium content of the extract was determined with flame atomic absorption spectrometer.

Results: Dry weights of roots and shoots decreased with increase in Cd concentration significantly. With increasing cadmium concentration from 2.5 to 15 ppm in the medium, shoot and root Cd concentrations were increased from 75 to 168 (µg.g⁻¹) and 1026 to 5656 (µg.g⁻¹), respectively.

Conclusions: *S. hortensis* is a tolerant plant to Cd which able to accumulate high amounts of Cd in their tissues. Further work is needed to examine whether the Cd treatment could also affect the *S. hortensis*'s secondary metabolites.

Keywords: Satureja hortensis, Cadmium, Accumulation.
Title:
Study on the interaction of curcumin with bovine alpha-lactalbumin

Authors:
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Department of Chemistry, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan, Iran

Abstract:
Introduction: Alpha-lactalbumin is the second major whey protein in bovine milk (2-5% of the total protein in bovine milk) and has significant role in lactose biosynthesis and milk production in the mammary gland. Alpha-lactalbumin binds divalent cations (Ca$^{2+}$, Zn$^{2+}$) and may facilitate the absorption of essential minerals and provides the essential amino acids for the growing infant. It has significant role in the protection against infection. Alpha-lactalbumin is an acidic, calcium metalloprotein, low molecular weight globular protein (14.2 kDa) with an isoelectric point of 4.6. Curcumin [bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], which has a strong yellowish color is extracted from plant turmeric (Curcuma longa). This polyphenol compound has anti oxidant, anti inflammatory, and anti-cancer properties and because of this, it has been used as a traditional medicine for treatment of inflammation and cancers in India and China. In this study, we investigated the binding interaction of curcumin with the milk protein alpha-lactalbumin. Method: In this study, spectroscopic techniques such as UV–Vis absorption, steady-state fluorescence, synchronous fluorescence, and molecular docking have been used. Results: The fluorescence titration experiments showed that curcumin has quenching effect on the fluorescence intensity of alpha-lactalbumin. The binding parameters including number of binding sites and binding constant have been calculated based on the fluorescence quenching data. The synchronous fluorescence results indicated that binding of curcumin may not cause considerable alterations in the conformation of alpha-lactalbumin. Conclusions: The results of this study indicated that alpha-lactalbumin not only has strong ability to bind divalent cations and fatty acids, but also can bind natural bioactive compounds like curcumin.

Keywords: Curcumin, Alpha-lactalbumin, Ligand binding
Title:
Study of the association between male infertility and rs2030259 polymorphic marker in JHDM2A gene

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Abstract:
Introduction: JHDM2A is a histone demethylase that specifically demethylates mono-and dimethylated histone H3 lysine9. JHDM2A (JmjC-domain-containing histone demethylase 2A, also known as JMJD1A and KDM3A) is essential for spermatogenesis. Also JHDM2A binds to and controls the expression of transition nuclear protein 1 (Tnp1) and protamine 1 (Prm1) genes, the products of which are required for packaging and condensation of sperm chromatin.

Methods: A non-synonymous SNP, rs2030259, has been selected by bioinformatics studies. Primers were designed by using oligo primer software. Genomic DNA was extracted from the blood of 100 patients with azoospermia and oligozoospermia, matched control and JHDM2A gene was amplified. Finally the samples analyzed by agarose gel.

Results: So far we have observed bands in considered length according to designed primers, for patient and control samples, and its relationship with male infertility is under investigation.

Conclusion: This study has focused on polymorphic marker of JHDM2A gene to evaluate its association with male infertility. The association reported in this study will be necessary to confidently validate this SNP and identify novel SNPs association with male infertility that can have therapeutic purpose.

Keywords: Histone demethylase1, JmjC-domain-containing histone demethylase2, Nuclear protein3, Protamine4.
Title:
Study on the interaction of genistein with hen egg white lysozyme

Authors:
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Affiliation:
Department of Chemistry, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan, Iran

Abstract:
Genistein (4,5,7-trihydroxyisoflavone), a natural isoflavone phytoestrogen found in soybeans and chickpea. The isoflavone genistein has been identified as having antiproliferative and apoptosis effects on various malignant cell types derived. Genistein is a potent agent in both prophylaxis and treatment of cancer as well as other chronic diseases. Chicken egg white lysozyme is one of the major egg white proteins with well demonstrated antimicrobial activity. Lysozyme has different physiological and pharmaceutical functions, so studies on the interaction of drugs and bioactive compounds with lysozyme are important for elucidation the relation of structure and function of lysozyme and also therapeutic effectiveness of drugs. In this study, we aimed to investigate the interaction of genistein as a natural isoflavone with chicken egg white lysozyme.

Keywords: Genistein, Lysozyme, Fluorescence Spectroscopy, Molecular Docking
Title:
Induction of callus culture in Artemisia sieberi L., a medicinal plant.

Authors:

Affiliation:
University of Mohaghegh Ardabili / Biology department of Mohaghegh university, Ardabil, Iran

Abstract:
Introduction: Artemisia sieberi is one of the most common medicinal plant which contains various compounds such as artemisin, camphor, α-thujon and β-thujon. These compounds are important because of their different use on food and pharmaceutical industry materials. Difficulty of multiplication of Artemisia sieberi in field based on important to thinking the research about multiplication by in vitro techniques like callus induction and tissue culture. Method: This research was done at tissue culture laboratory of plant biology department of Mohaghegh university. Seeds of Artemisia sieberi were cultured on different concentrations of MS medium: MS, 1/2MS and 1/4MS. After seed germination, root segments and stem segments of the seedlings were transferred to MS medium containing various mixture of 2,4-D and Kinetin hormones with different concentrations. Results: After two weeks our results showed that callus tissue was grown in all of MS medium with different hormonal concentration. Although, in the hormone less MS medium callus growth was observed, the maximum callus production take placed in the MS medium with 0.5 mg/L of 2,4-D and Kinetin and we had the most green and yellow calluses. Conclusions: It can be concluded that Artemisia sieberi callus culture growth can be induced in MS medium.

Keywords: Artemisia sieberi, Artemisin, Tissue culture, Callus induction.
Title: 
Clinicopathological relevance of the expression of Eyes Absent Homolog 1 gene in gastric carcinoma

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P. Nikpour 1, 2, 3, *, E. Emadi-Andani 4, M. Emadi-Baygi 5, 6, S. Rahmati 5

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Abstract: 
Introduction: Gastric cancer is the second and fourth most common cancer in Iranian men and women respectively but it is the first leading cause of cancer deaths in Iran. Most Iranian patients with gastric cancer are diagnosed at an advanced stage of disease when the conventional treatments have no effect on improving the survival. So, early gastric cancer detection using new molecular markers is of high priority in order to decrease its high mortality rate in Iran. The Eyes Absent (EYA) proteins are implicated in processes as disparate as organ development, innate immunity, DNA damage repair, angiogenesis, and cancer metastasis. EYA1, a member of this family, is shown to overexpress in several tumor types like Wilms' and neuroblastic tumors. The aim of this study was to evaluate the clinicopathological relevance of the expression of EYA1 gene in gastric carcinoma. Method: A total of 60 tumoral and non-tumoral gastric specimens were evaluated for EYA1 gene expression using quantitative real-time PCR. Results: The expression of Eyes Absent Homolog 1 was heterogeneous in gastric specimens. We further showed that there was a positive correlation between the EYA1 gene expression and patient age, but not with other clinicopathological features of gastric tumors, like sex, N and M classification, lymphatic invasion and tumor size. Conclusions: Eyes Absent Homolog 1 could serve as a tumor marker in gastric carcinoma. In future works, the mechanism by which EYA1 affects gastric tumorigenesis must be evaluated.

Keywords: Gastric cancer, Eyes Absent Homolog 1, quantitative real-time RT-PCR
Title:
Virtual analysis Inhibitory Effects of herbal ingredients on Hepatitis C Virus (HCV) Protease

Authors:
Seyed Dawood Mousavi Nasab 1 Absalan Abdolrahim 2 Farzaneh Sabahi 1 Mehrdad Ravanshad* 1 Masoud Hamidi 3 Fatemeh Zali 2

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Abstract:
Introduction: The current normal therapy for chronic hepatitis C patients is a pegylated interferon (IFN)-α, and combination either or alone with ribavirin. This therapy protocol causes sustained virological response in only 50% of infected patients. Limited efficacy and adverse side effects of the current therapies has clearly demonstrated that there is an awful need to develop more effective antiviral agents. Natural products, especially plants have been used for the treatment of various diseases for thousands of years and are as an alternative to conventional chemical agents. So far a number of herbal extracts have been screened for their antiviral effect against many HCV infections. In the current study, we have assessed interaction of 7 chemicals, from which 5 are natural ingredients in botanical products and two are synthetic products approved by FDA for HCV therapy, with NS3 protease.
Method: Virtual analysis of NS3 docking with 5 herbal ingredients including Epigallocatechin gallate, Curcumin, Astragalin, Picrocrocin, Cytisine and two therapeutic drugs, Telaprevir and Boceprevir. Molegro virtual docker 2010.4.2 (CLC bio) is used for docking of chemicals with NS3 protein. Docking results are analyzed statistically for analysis of variance (ANOVA) and Games-Howell for POST-HOC multiple comparisons using spss software ver.16.
Results: Docking results shows that mentioned herbal ingredients have significant differences for their interaction energy with NS3-4A protein according to the ANOVA statistic (CI=0.95; P=0.000).
Conclusions: Results suggest that molecular basis of the Natural products could play a great inhibitory activity sources for HCV agents and could apply in optimization and subsequent development of specific antiviral agent.

Keywords: HCV NS3 protease, antiviral agents, herbal ingredients, docking
Title: Antioxidant and Free Radical Scavenging Effects of Curcumin on ROS-induced Cell Death in SK-N-MC Cells

Authors: Maryam Kamarehei and Razieh Yazdanparast

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Abstract: Introduction: Oxidative stress has been implicated to play a vital role in the pathogenesis of neurodegenerative disorders including Alzheimer’s disease. Reactive Oxygen Species (ROS) are highly reactive with biomolecules. Collectively, ROS can lead to oxidation of proteins and DNA, peroxidation of lipids and ultimately cell death. Recent studies have suggested positive effects of antioxidants as an aid in potentially reducing neuronal damage caused by free radicals. Curcumin, a yellow pigment extracted from rhizome of the plant *Curcuma Longa*, has been shown to have antioxidative properties. Methods: Hydrogen peroxide and menadione (superoxide anion producer) were used to induce oxidative stress in SK-N-MC neuroblastoma cells then acridine orange/ethidium bromide double staining method was applied to observe apoptotic cells. Free radical scavenging potential of curcumin was investigated through MTT assay and antioxidant enzymes activity assay. The extent of lipid peroxidation, protein oxidation and also ROS levels were evaluated as biomarkers of oxidative stress. Results: Our studies showed that pretreatment of the cells with curcumin reduced ROS-induced apoptosis. It also decreased lipid peroxidation, protein carbonyl formation and ROS levels induced by hydrogen peroxide and menadione and restored catalase activity. Conclusions: Collectively, menadione scavenging potential of curcumin was more effective than its hydrogen peroxide scavenging potential. Comparatively speaking, our present data indicated that curcumin can be a promising candidate in antioxidant therapy and designing drugs for ROS-induced neurodegenerative diseases.

Keywords: Oxidative stress, Neurodegenerative disorders, Reactive Oxygen Species, Antioxidant therapy
Title:
Host genes of Clustered microRNAs as a Potential Target for other miRNAs

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Abstract:
Introduction: microRNAs are small noncoding RNAs which post transcriptionally regulate gene expression. These molecules are important components of gene regulating networks. As a clustered microRNA, MIR17HG is comprised of at least six miRNAs. In this study, we aimed to predict miRNAs that have the ability to bind and regulate the expression of MIR17HG locus.

Method: The putative miRNA-mRNA pairs were identified by using various prediction softwares including miRanda, RegRNA, TatgetScan, PicTar and miRWalk. miRNAs which were predicted to target the MIR17HG locus in all softwares were selected.

Results: This method of miRNA target prediction could reduce the number of interacting miRNAs with MIR17HG locus from several hundred miRNAs to about 150 ones. Therefore, this method may facilitate experimental validation of in silico miRNA target predictions.

Conclusions: Since these clustered miRNAs are considered as oncogenic miRNAs in a number of cancers, the miRNAs which are targeting this cluster may act as tumor suppressors in these cancers and maybe used as cancer targeted therapies in future.

Keywords: MIR17HG, microRNA (miRNA), target prediction, miR-mRNA
Title: Association of the length of polyalanine tract in the transforming growth factor β receptor 1 gene & risk of breast cancer

Authors: Elahe Kamali*, Manoochehr Tavassoli¹, Parisa Kheradmand¹, Simin Hematti², Foroozan Safari¹
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Abstract: Introduction: Breast cancer is a disease resulting from complex interactions between environmental and genetic factors. Genetic factors such as genetic polymorphisms could modulate several important biological progress and alert susceptibility to cancer consequently. TGF-β is a multifunctional cytokine belonging to the TGF-β superfamily of secreted cytokines that plays a complex role in breast carcinogenesis. Initially TGF-β acts as a tumor suppressor by inhibiting cell proliferation, but as tumor progression occurs, malignant cells become resistant to its growth inhibitory effects. Aberrations of the TGF-β signaling pathway are frequently found in many diseases including human cancers in breast, colon, prostate or pancreas. TGFBR1 contains a common alanine coding region with a GCG repeat. Polymorphism in this region may be attributed to breast cancer. The purpose of this study is the evaluation of this polymorphism to clarify whether there is any association between them and breast cancer risk.

Method: This study was a case-control study on 150 patients and 150 controls women. After DNA extraction from the blood sample of study subjects, the polymorphism expansion was amplified by the technique of Polymerase Chain Reaction (PCR). Thereafter the number and sequence of GCG was analyzed by polyacrylamide gel and direct sequencing. Results: So far we have observed alleles with different number of GCG repeats. The results of this study were shown that TGF-βR1 gene allele distribution varies between 6 to 9 repeat and (GCG)9 was the most common allele between cases and controls. Women with (GCG)6 genotype may be in a greater risk of breast cancer.

Conclusions: These findings indicate a direct relationship between the number of repetitive sequence in exon1 of TGF-βR1 gene and increased risk of breast cancer.

Keywords: Breast cancer, TGF-βR1, GCG repeat, Polymorphism
Title: Evaluation of effect of Positive Pressure Ventilation on Pharmacokinetic behavior of serum level of Phenytoin in Traumatic Brain Injury patients

Authors: Mahmoodpoor A1, Hamishhekar H2, Farhoodi Sh3 1-Department of Anesthesiology and Intensive Care Medicine 2-Department of Clinical Pharmacy, 3-Students Research Committee Tabriz University of Medical Sciences

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Abstract: Introduction: Previous studies have suggested that drug metabolism may be altered in severe neurotrauma patients. Also positive ventilation has shown to have an influence on pharmacokinetic and deposition of some drugs by decreasing of cardiac output, glumerular filtration and hepatic blood flow, specially, drugs with hepatic or renal clearance. In this prospective randomized clinical trial study, we want to evaluated the effect of PEEP(Positive End Expiratory Pressure)(a biophysical item) on pharmacokinetic and serum level of phenytoin(a biochemical item) in neurotrauma patients that receive phenytoin as seizure prophilaxis. Method: 36 patient with acute traumatic brain injury who were admitted to intensive care unit and received phenytoin for sizer prophilaxy enrolled in this trial and randomized into two groups, (group A, 20 patient with GCS <8 who need PEEP (5- 10 cm H2O) and group B, 16 patient with GCS>8 who didn't need PEEP. Free fraction, total serum level of phenytoin and pharmacokinetic parameters were measured. Samples were collected at 72 hours after first phenytoin administration and 30 minutes before the next dose(through concentration). Patient received phenytoin infusion with a loading dose of 12–15 mg /kg in 15 minutes via microset which is followed up by maintenance dose of 3–7 mg /kg in 3 divided doses. (completion of 9 doses). Michaelis-Menten pharmacokinetic model was used to calculate V max and clearance for each patient. Statical analysis was performed by SPSS 11.5-13. Result: Clearance, V max and drug dose for each patient was calculated. V max in group A was 5.72±0.62 and group B was 5.53±0.62 mg/kg/day. Clearance in group was 0.28±0.11and 0.25±0.13 l/kg/day in group A and B respectively. Free fraction, total serum level and free to total ratio was measured for each patient. Phenytoin free fraction in group Awas 0.07±0.04 and in group B was 0.05±0.01 with Pvalue<0.005 which showed statically significant differences between two groups. Coclusion: Our data showed that there is statistically significant differences in the calculated V max ( Pvalue : 0.37 ) and clearance ( Pvalue : 0.47 ) between groups . receiving equal dose of phenytoin, serum level of it have significant difference , This matter is cleared by limited metabolism rate of drug difference in V max in patient variation in serum Albumin level . Keywords: PEEP, phenytoin, neurotrauma, seizure, pharmacokinetic
Title:
The Study of Hydrogen Bonds Between Curcuminoids and C1B Subdomain of Protein Kinase C (PKCδC1B) By Docking Method

Authors:
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Abstract:
Introduction: Protein kinase C (PKC) is a family of serine/threonine protein kinase. PKC isoforms play important role in the pathology of several diseases such as cancer, diabetes and Alzheimer’s disease. Therefore, PKC has been a subject of intensive research and drug development. The C1 domains of PKC have become an attractive target in designing the its based drugs. Recently it was found that curcuminoids bind to the C1 domain and modulate PKC. A curcuminoid is a curcumin or its derivatives with different chemical groups. Curcumin has anti-inflammatory, anti-oxidant properties. Animal studies have suggested that curcumin may be active against a wide range of human diseases, including diabetes and cancer. There are some studies about recognizing how PKC is modulated by curcumin in vivo and in vitro, but there isn’t any theoretical study about it, so, we investigate interactions of Curcuminoids with C1B domain of PKCδ by computational methods. Method: Autodock 4.2 software was used for docking analysis. After the simulation was complete, the docked structures were analyzed. Hydrogen bond interactions and the binding distance between the donors and acceptors were measured for the best conformers. Result: Which residues that participate in hydrogen bonding interactions were listed in table 1

<table>
<thead>
<tr>
<th>curcuminoid</th>
<th>residue participated in hydrogen bonding interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keto isomer of curcumin</td>
<td>LEU259, HIS270, TRP252, TYR238, GLY258</td>
</tr>
<tr>
<td>Enol isomer of curcumin</td>
<td>GLY258, LYS260, TYR238, THR242, ASN267, GLY258</td>
</tr>
<tr>
<td>Bisdemethoxycurcumin</td>
<td>LYS260, ASN267</td>
</tr>
<tr>
<td>Bisdehydroxycurcumin</td>
<td>No hydrogen bonds formed</td>
</tr>
</tbody>
</table>

Conclusion: Methoxy group specially hydroxyl group of curcumin are essential for forming hydrogen bond of curcumin with amino acids of C1B domain of PKCδ

Keywords: curcuminoid, protein kinase C, Autodock
Title:
A novel enzymatic method for silver nanoparticles synthesis mediated by α-amylase from Bacillus methylotrophicus

Authors:
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Abstract:
Introduction: Preparation of nanometals using physicochemical methods supply nanostructures with narrow and controlled size ranges, however, these methods require very expensive equipments and the final yield is low, for this reasons studies on the improvement of biological techniques for synthesis of nanoparticles have been extensively increased. Silver nanoparticles (AgNPs) are applied as selective coating agent for solar energy absorption, intercalation material for electric batteries, catalysts in chemical reactions and antimicrobial agents.

Methods: Formation of silver nanoparticles was investigated by using of the purified enzyme from Bacillus Methylotrophicus followed by adding aqueous concentrations of AgNO3 (0.05 to 10 mM) with different mole ratios (1:1, 1:2, 1:4, 1:5 and 1:10). The prepared mixtures were incubated at different temperatures (50°C-70°C) and we saw black sediment after 72 hour. UV-Vis spectra of the mixtures of nanoparticles were recorded in the range of 300–700 nm. Other characteristics of produced nanoparticles such as average particle size and FTIR were also determined.

Result: UV-Vis spectrum for Ag solution after incubation showed peaks at 430 nm. Biosynthesis of AgNPs occurred at 50 and 70°C in 5 mM concentrations of AgNO3 with ratios of 1:2, 1:4 and 1:10. Optimization of silver nanoparticle synthesis was also carried out. The average particle sizes of nanoparticle analyzed. Results showed that glucose accelerated the nanoparticle synthesis.

Conclusions: The ability of α-amylose and probably its reducing group as a green biocatalyst for production of silver nanoparticles were presented.

Keywords: Keywords: Silver nanoparticle (AgNO3); Bacillus Methylotrophicus, Alpha amylase, FTIR,
Title:
Effect of electromagnetic field on enhancement of ferritin content in staple food crops

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Abstract:
Introduction: One of the interesting bio-molecules in biological systems is ferritin. Ferritin is a reserving protein that accumulates Fe\textsuperscript{2+} ions in living organisms. Since iron is an important micronutrient for human and about 2 billion people worldwide suffer from iron deficiency anemia (IDA), therefore, it's necessary to change dietary diversity and utilize staple crops with high levels of bioavailable iron. Biofortification of crops is one of the programs to achieve this goal. On the other hand, study on biological effects of magnetic and electromagnetic fields are growing up recently. On the base of Ion Cyclotron Resonance (ICR) theory, application of low frequencies of electromagnetic field which can accelerate ions in a cyclotron, can increase movement of bio-molecules in cells and improve the growth

Method: In this study, soybean and maize seeds were exposed to a designed combination of alternative electromagnetic and geomagnetic fields with cumulative intensity of 20\textmu T for 4 days. after that seedlings transferred to Hogland nutrient solution for 6 days. Ferritin were extracted from seeds, shoots and roots of plantlets and it's content were measure with ELIZA method.

Results: According to the results, growth rates of both plants and their ferritin content significantly increased by exposure to the electromagnetic field.

Keywords: Electromagnetic field, ELIZA, Ferritin, Maize, Soybean.
Title:
Exploring methylotrophic yeast Pichia pastoris as a platform for expression of cell surface adhesive proteins

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Abstract:
Introduction: The C-terminal repeat region of peptidoglycan hydrolase (CPH) of Lactococcus lactis IL1403 produced intracellularly in Escherichia coli was able to attach to the cell surface of lactic acid bacteria (LAB) when added from the outside. Therefore, CPH can be used for binding of a protein of interest to LAB cells when the fusion of CPH to the target protein is incubated with the cells. These cell surface adhesive proteins can confer new functions to LAB without making any genetic modifications in them and are valuable for food and vaccine development. Pichia pastoris has better capability to allow the correct folding of recombinant proteins than prokaryotic hosts e.g. E. coli. However, the glycosylation of the proteins in this yeast may affect their functions. In this study, therefore, we investigated expression of a CPH mutant devoid of potential N-glycosylation sites (CPHM) in P. pastoris. The cell surface binding activity of CPHM was studied and compared with that of CPH produced in E. coli.

Methods: cph was cloned into pPICZalphaC (invitrogen) with a hexa histidine tag encoding sequence at its N-terminus. The resultant plasmid was pPlalphaCPH. For construction of CPHM, site directed mutagenesis was performed using a Quickchange kit (Stratagen), three mutagenic primers and pPlalphaCPH as the template. Protein expression in P. Pastoris GS115 was induced with methanol at 0.5% v/v. Protein purification was done using nickel chelate affinity chromatography. Protein expression in the yeast and binding of proteins to the cell surface was studied by western blot.

Results: CPHM was successfully expressed extracellularly in P. pastoris using alpha-mating factor signal sequence, whereas the native CPH was not produced in this host. Western blot analysis revealed that the apparent molecular size of CPHM was greater than that of CPH produced in E. coli, which is attributed to O-glycosylation. However, CPHM produced in P. pastoris was capable of binding to the cell surface of L. casei NRRL B-441 despite its modification by the yeast, and its dissociation rate constant from the cell surface was 3.5-fold lower than that of CPH produced in E. coli.

Conclusion: These results demonstrate the applicability of the constructed domain (CPHM) for the production of cell-surface adhesive proteins in P. pastoris.

Keywords: cell surface adhesion, glycosylation, lactic acid bacteria, peptidoglycan hydrolase, Pichia pastoris
Title: Molecular modeling and docking of some coumarin derivatives as β-secretase inhibitors

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Abstract: Introduction: Development of new potential class of drugs to block β-secretase would stop the build up β-amyloid and may help to prevent Alzheimer’s disease. In order to establish the possible potential drugs to obstruct β-secretase, a series of twenty four 4-methylcoumarin derivatives have been examined by employing computational molecular docking approach. This method is a process which predicts the favorite orientation of ligand molecule to receptor when bound to each other to form a stable complex. Method: The geometry of twenty four 4-methyl coumarin derivatives have been optimized considering the molecular dynamic simulations and followed quantum computational semi-empirical molecular orbital theory at the level of AM1 by the help of HyperChem8 and Gaussian98. The optimized structures used for molecular docking studies via autodock 5.4. Docking studies of 4-methyl coumarin derivatives have been carried out in the active site of β-secretase. Results: Data indicated that all of 4-methylcoumarin derivatives have affinity to bind the active site of β-secretase. However, some of the 4-methylcoumarin derivatives had a greater binding affinity because of appropriate H-bonds formation with Asn and Lys/Arg residues at the active site of enzyme suggest the suitable candidates to inhibit β-secretase. Additionally, the results denoted that the molecular size, the size of substitution in position R3 and type of substituents at the position of R5/ R7 of 4-methyl coumarin were related to the binding affinities of β-secretase active site. Conclusion: Hydrogen-bonding and hydrophobic interactions were observed to be characteristic interactions between 4-methylcoumarins and β-secretase active site that imply the affinity and activity of the molecules. The findings would be useful for therapeutic development of Alzheimer’s disease.

Keywords: Alzheimer, β-secretase, 4-Methylcoumarin, Docking, MD simulations
Title:
The Interaction of Fisetin as Flavonoid with Beta Lactoglobulin: Docking and Molecular Dynamics Simulation Studies

Authors:
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Abstract:
Flavonoids represent a group of phytochemicals exhibiting a wide range of biological activities arising mainly from their antioxidant properties and ability to modulate several enzymes or cell receptors. Flavonoids have been recognized to exert anti-bacterial and anti-viral activity, anti-inflammatory, anti-angionic, analgesic, anti-allergic effects, hepatoprotective, cytostatic, apoptotic, estrogenic and anti-estrogenic properties. Beta-Lactoglobulin is the major whey protein in the milk of ruminants and some nonruminants, such as pigs and horses. Recent x-ray crystallographic studies have advanced knowledge of the structure of beta-lactoglobulin, which is homologous with that of retinol-binding protein and lipocalycins; the function of these proteins seems to be participation in the transport of small hydrophobic substances, ligands, drugs and vitamins. By analogy, this protein has been suggested as having a role as a transporter of fatty acids and retinol that this property has twice the beta- Lactoglobulin value. Given the importance of flavonoids and beta-Lactoglobulin protein transport properties the interaction of these two compounds is important. The interaction of Fisetin as Flavonoid with beta-Lactoglobulin using molecular docking (AutoDock software) and molecular dynamics simulation (Gromacs software) methods was examined. Finally, we use the results of the protein (BLG) ability to make appropriate bound with flavonoid ligand and its transmission will follow.

Keywords: Flavonoid, Beta-Lactoglobulin, Fisetin, Molecular Docking, Molecular Dynamics Simulation.
Title:
Dynamic Light Scattering Study Of Miniapoptosome Formation

Authors:
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Abstract:
Introduction:
Intrinsic apoptosis pathway causes the activation of procaspase-9 through the release of cytochromeC from the mitochondria. In the cytosol, cytochromeC binds the adaptor molecule Apaf1 (apoptotic protease activating factor-1) comprised of an N-terminal caspase recruitment domain (CARD), a nucleotide binding/oligomerization domain, and C-terminal WD40 repeats. In lower eukaryotic organism, C.elegans, CytochromeC does not play any role because its Apaf-1 lacks the cytochrome binding domain. A complex formed by the interaction of caspase-9 and the Apaf-1 lacking WD40 domain is called miniapoptosome. It has been proposed that the formation of functional miniapoptosome requires both ATP or dATP nucleotide exchange and the presence of caspase-9. In this study we aim to assess the validity of the above-mentioned hypothesis.

Methods:
Recombinant Apaf-1 (1-591) and caspase-9 proteins were over expressed in BL21DE3 and purified to homogeneity using Ni-NTA column (Qiagen). In order to assess whether or not Apaf-1 was functional, Caspase-9 activation Assays were setup in the presence and absence of various concentrations of ATP. The cleavage of caspase-9 substrate LEHD-PNA was monitored at 405nm. In addition Dynamic Light Scattering was used to determine the size of the complex under various conditions.

Results:
Our results showed that the freshly produced Apaf-1 was active without the addition of ATP or dATP. Moreover, higher concentrations of dATP exhibited an inhibitory effect on the caspase-9 enzymatic activity. Furthermore, fresh Apaf-1 formed a complex with approximate hydrodynamic diameter of 80nm. Upon storage at -70, Apaf-1 lost its ability to activate caspase-9 and its hydrodynamic diameter was reduced to 14nm. Addition of ATP or dATP did not restore Apaf-1 activity.

Conclusion:
Our results show that the nucleotide exchange is not required for Apaf-1 activation and it seems that the purified protein already possessed the nucleotide. In addition, functional apoptosome can be formed in the absence of caspase-9.

Keywords: Miniapoptosome, Apaf-1, Dynamic Light Scattering
Title:
Study the effect of seed priming by plant growth regulators on some biochemical criteria in Matricaria aurea

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Abstract:
Introduction: Currently the use of plant growth regulators for enhancement of seed germination and seedling growth criteria has been increased. In order to investigate effect of seed priming by Salicylic acid and Ascorbic acid as plant growth regulator on seed germination and seedling growth of Matricaria aurea we must study some biochemical criteria.
Method: In this case a completely randomized experiment with four replication was conducted in plant physiology laboratory of science faculty of Mohaghegh-e-Ardabili university. Experimental treatments include optimum concentration of Salicylic acid and Ascorbic acid for germination.(150ppm from Salisylic acid and 50ppm from Ascorbic acid.) Result revealed that seed priming by 150ppm from Salisylic acid and 50ppm from Ascorbic acid had enhanced some biochemical criteria such as total soluble sugars and proteins in comparison to control. Also other biochemical experiments are attempting.
Conclusion: With observation an enhancement in some biochemical criteria, we can utilize some special plant growth regulators to improve seed germination and seedling growth criteria of medical and useful plants.
Key words: Ascorbic acid, Salisylic acid, Matricaria aurea, Total soluble sugar, Soluble proteins.

Keywords: Ascorbic acid, Salisylic acid, Matricaria aurea, Total soluble sugars, Soluble proteins.
Title:
An Improved Immunosensor to detect Witches' Broom Disease (WBD) of Lime

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Abstract:
Introduction: WBD is a disease to affect small-fruited acid limes which almost kills the trees within a few years. Infected trees contain many microorganisms in their sieve tubes which surrounded by a single cell membrane, without cell wall. They are similar to the mycoplasma-like organisms (MLO) and named "Candidatus Phytoplasma aurantifolia". In Julay 1997, the disease was observed in the southeastern region of Iran and many gardens have been destroyed up to now. Although PCR and enzyme-linked immunosorbent assay (ELISA) are used to detect infected trees but in this project we used a new immunosensor that is based on a specific antibody against the pathogens in infected trees.

Method: Silicon substrates from microfabricated readout system were silanized using 3-aminopropyl-triethoxysilane (APTES) and glutaraldehyde used as a linker. Each step of the surface preparation was controlled and optimized using FT-IR. Then the antibody against P. aurantifolia was verified using ELISA and immobilized on activated silicon substrates to detect antigens in the extract of samples.

Results: An appropriate microfabricated electronic devise was created and FT-IR showed its silicon surface was successfully functionalized with anti-WBD antibodies. Based on our knowledge, this is the first report that demonstrates a proof-of-concept that antibody coated piezoelectric sensors can be used to quantify of antigens at law picogram per milliliter levels in samples.

Conclusions: Our results demonstrated that this immunosensor can be developed as a new strategy for high throughput detection of plant pathogens. This approach may be expanded to other antibody-based detection in agricultural and medical fields.

Keywords: Piezoelecteric immunosensors, Withes' broom dieasease (WBD), Silanization, 3-aminopropyl-triethoxysilane, Enzyme-linked immunosorbent assay (ELISA).
Title:
Isolation and characterization of ferritin nanoparticles of wheat seedlings exposure to static and electromagnetic fields

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Abstract:
Effect of static magnetic field (SMF) and electromagnetic field (EMF) on plants have been investigated for more than three decades, but the exact mechanism of these effects is poorly understood and there is no adequate literature on the application of these fields in order to development of strategic plants. Ferrimagnetic resonance model is one of major mechanisms of magnetoreception by living organisms. In this model, the magnetic signal is coupled to the magnetization vector, causing it to resonate as if the particle’s size and shape are consistent for resonance at the transmission frequency. Ferritin, a storage protein with a highly conserved structure can store up to 4500 iron atoms in a non-toxic and biologically available form. In the present study imbibed seeds of wheat (Triticum aestivum L.) were treated with SMF (30 mT) and EMF (10 kHz) for 4 days, each 5 hours. After isolation of ferritin nanoparticles from seedlings, their content (using ELISA method), structural changes (using circular dichroism spectroscopy) and size (by monitoring the change of hydrodynamic diameter using a Zetasizer instrument) were assayed. Treatment with EMF and SMF decreased significantly the content of ferritin of wheat seedlings compared to the control groups. The secondary structural content of ferritin significantly increased and decreased by SMF and EMF treatments, respectively. In comparison with the control seedlings, Z-average of ferritin nanoparticles was higher in EMF-treated seedlings but lower in SMF-treated ones. The results can explain the mechanism of perception of MF by plants in the frame of ferrimagnetism model.

Keywords: EMF, ferritin nano particles, magnetic field, SMF, wheat
Title: Production of active recombinant human growth hormone in escherichia coli strain rosetta-gami

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Abstract: Introduction: Human growth hormone (hGH) is a small, single chain peptide 191 residues, produced and secreted by the anterior pituitary gland. It is one of the most important hormones in the human body due to its pivotal role in a variety of biological functions. This protein is responsible for effects in metabolism of proteins, carbohydrates and lipids as well as in growth, development and immunity. Growth hormone is used as a prescription drug in medicine to treat children’s growth disorders, adult growth hormone deficiency, Turner’s syndrome, Prader-Willi syndrome, chronic kidney insufficiency and short bowel syndrome. The main source of GH for human use is recombinant GH (rhGH) production by bacteria. Rosetta-gami host strains are Origami derivatives that combine the enhanced disulfide bond formation resulting from trx/gor mutations with enhanced expression of eukaryotic protein. In this study, we report production of recombinant human growth hormone in the *E. coli* strains Rosetta-gami.

Method: *E. coli* Rosetta-gami competent cells were transformed by plasmid containing human growth hormone gene and cultured in LB medium. After induction by IPTG, recombinant human growth hormone production was assessed using dot blotting, western blotting and ELASA, rhGH was purified using affinity chromatography and its biological activity was assessed using reporter gene assay by means of luciferase activity.

Results: rhGH was produced successfully at high levels in *E. coli* strains Rosetta-gami. These bacteria could be used for production of rhGH at industrial levels.

Conclusions: The biological activity of rhGH produced, in this study has been found comparable with the available commercially version.

Keywords: *E. coli* strains Rosetta-gami, western blotting, recombinant human growth hormone.
Title:
Investigation of suicide inactivation of Heme- Tosyl Imidazole-SDS as a peroxidase like artificial enzyme

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Abstract:
A ternary complex consisting of Heme – 1-tosyl-1H-imidazole (TsIm) – SDS has been designed as an artificial peroxidase enzyme which demonstrated the catalytic efficiency about 26.38% relative to native Horse Radish Peroxidase. All Hemoproteins including peroxidases are inactivated at high peroxide concentration. Suicide inactivation of the peroxidase like artificial enzyme (Heme – TsIm – SDS) followed at 470 nm by difference spectrophotometry during a time course of 10 minutes. Monitoring the concentration of AH (reducing substrate) through the catalytic cycle can lead to estimation of the extent to which the active enzymatic form (compound I) decreases as result of suicide inactivation process. The result obtained by fitting the experimental data to the overall integrated kinetic equations. It was an excellent agreement between experimental data and the curve fit which was based on the similarity of the suicide mechanism of HRP and the biocatalyst. The k parameter for the Heme – TsIm – SDS is less than k of native HRP. It shows that presence of TsIm as a donor ligand significantly decreases the rate of inactivation because TsIm can distribute electron charge in the structure of the active form of the biocatalyst (compound I) via conjugation and hyper conjugation. In fact alkyl groups as the electron donors can make the structure of the ion radical better to resistance in the high concentration of Hydrogen peroxide compare to native HRP.

Keywords: artificial peroxidase enzyme, HRP, Tosyl Imidazole (TsIm)
Title:
Polymorphic variants of the interferon- gamma gene in tuberculosis patients

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Abstract:
Introduction: Cytokines play an important role in anti-mycobacterial response and may determine the type of tuberculosis. Thus, gene polymorphisms associated with cytokine production may be associated with susceptibility to TB. The objective of our study was to analyze the frequency of cytokine gene polymorphism (INF-γ [UTR]5644 T/A) in tuberculosis patients.

Methods: This case-control study performed on 100 patients with tuberculosis and 194 healthy blood donors. DNA of blood sample of the people was purified by DNA extract kit, then cytokine gene polymorphism interferon-γ [A/T untranslated region (UTR)5644] was determined by cytokine gene polymorphism SSP kit (Heidelberg University, Heidelberg, Germany) and PCR-SSP method. The product obtained of PCR on agarose waselectrophoresed.

Results: We genotyped 100 patients (50 Pulmonary, 50 Extrapulmonary TB) and 194 healthy blood donors. We observed significantly decreased frequency of genotype of INF-γ (A/T UTR 5644) in PTB patients compared to the control and PTB groups (p=0.03) and T/T homozygous genotype increased in PTB. Also genotype frequencies were higher in PTB than control (P= 0.016). There was no difference in EPTB and control groups (P=0.48). Frequencies of high producing INF-γ (UTR 5644) T allele were significantly over-represented in PTB group in comparison to both control and EPTB groups.

Conclusion: Our results suggest that INF-γ high-producer polymorphism is associated with PTB. INF-γ genes Polymorphism play key roles insusceptibility to or protection against tuberculosis development in the Iranian population.

Keywords: Tuberculosis, polymorphism, INF-γ
Title:
Molecular diagnosis of Leber congenital amaurosis by use of linked polymorphic markers in the Iranian population

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Abstract:
Introduction: Leber congenital amaurosis (LCA) is the most severe form of inherited retinopathy in humans. LCA is genetically heterogeneous and inherited in an autosomal recessive manner. Aryl hydrocarbon receptor interacting protein-like 1 (AIPL1) gene has been identified as the major cause of the disease. Sequencing followed by direct PCR has been used to detect point mutations and other sequence variations in the gene. Alternatively cosegregation analysis of polymorphic markers such as single nucleotide polymorphisms (SNPs) has been used in molecular diagnosis of the disease.

Material and methods: In the present study the possibility of using polymorphic markers presented in NCBI electronic database was examined. Two SNP markers were selected through bioinformatics investigation of the AIPL1 gene region. Primers were designed by using Oligo primer software. Genomic DNAs were genotyped using specific primers.

Results: The heterozygosity rate, allelic frequency and linkage of the markers, rs11658369 and rs8066853, were investigated. The results indicated the presence of linkage disequilibrium between the markers and the AIPL1 gene.

Conclusion: Together, the data suggest that rs11658369 and rs8066853 markers could be used as appropriate markers in the AIPL1 gene region in molecular and prenatal diagnosis of LCA disease in the Iranian population.

Keywords: AIPL1; Leber congenital amaurosis; polymorphic markers; linkage; Iranian population
Title:
Bioinformatics applications of Amino Acid-NTRU-Crypt Algorithm in a hybrid cryptography system

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Abstract:
Introduction: With the growth of Internet, providing robust security on messages through public channels is difficult. The symmetric and asymmetric encryption schemes by using a key and public/private key pairs respectively are known to be the best ways for this purpose.

Material and method: In this study, a new cipher algorithm, a combination of symmetric and asymmetric systems, is presented. In the symmetric section, the bioinformatics applications of biological processes (i.e., Amino acid protein sequences) are taken into consideration by mapping the message into the amino acid sequence including all 26 characters (i.e. including 20 amino acids and 6 extra special amino acids) used for random key generation and a lookup table containing amino acid combinations. The encoded amino acid message and the corresponding key will then be encrypted by NTRU public key crypto system which is a fast and easy implemented asymmetric cipher. Moreover, a safe protocol is essential for sending and receiving the encrypted message using public/private key for encryption and decryption process. By decrypting the NTRU cipher message the symmetric key and encoded amino acid protein will be revealed.

Results and discussion: This cipher is one of the fastest hybrid ciphers that its complexity of encryption and decryption is $O(n^2)$. For breaking the NTRU and the symmetric cipher, a lattice matrix with the order of $O(2n^2n)$ and brute force attack with the order of $26!$ are needed respectively.

Conclusion: The overall complexity of breaking this cipher is $O(26!*4n^2)$. So the hybrid cipher system comprising of NTRU and encoded amino acid sequences is remained unbreakable.

Keywords: Amino acid sequence, Bioinformatics, Protein, NTRU, Symmetric and asymmetric cipher
Title:
Study the interaction between adenosine deaminase and cationic surfactants by molecular dynamics

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Abstract:
Adenosine deaminase (ADA) catalyzes a rapid and irreversible conversion of adenosine to inosine this enzyme is able to deaminate a wide range of structurally diverse purine nucleosides and plays a key role in purine metabolic pathways as well as in mammalian immune system development. ADA, an enzyme distributed in the human tissues, is considered a good marker of cell mediated immunity. We want to study the effects of cationic surfactants with different concentrations on the activity of adenosine deaminase by molecular dynamics. For obtaining complementary information about the structure of ADA and influence of different concentration of cationic surfactant on it, MD calculations were performed on it. The used surfactant include of dodecyl trimethyl ammonium bromide (DTAB) and octyl trimethyl ammonium bromide (OTAB). The radius of gyration ($R_{gyr}$) curves and surface area of ADA in presence of different concentrations of OTAB and DTAB has been obtained. According to these results radius gyration diagram of ADA by molecular dynamics in the presence of low concentration of DTAB or OTAB is lower than high concentration of them. Also we abtain with increasing the concentration of surfactant, area of protein increases. So ADA has been unfolded more at high concentrations of OTAB or DTAB.

Keywords: Adenosine deaminase, molecular dynamics, cationic surfactant, DTAB, OTAB
Title: Kinetic studies on the proteinase K in the presence of Guanidine hydrochloride


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Abstract: Introduction: Proteinase K (EC 3.4.21.14) from the fungus Tritirachium album Limber is the most active known serine endopeptidase. A specificity of the enzyme for peptide bonds adjacent to the carboxylic group of aliphatic and aromatic amino acids was observed. The enzyme belongs to the subtilisin family. It can readily hydrolyze native proteins and remains active in the presence of urea and sodium dodecyl sulfate. Guanidinium chloride widely used as protein denaturants, Therefore activity and conformation of enzyme is change in the presence of Guanidine hydrochloride. We study the effect of increasing concentrations of guanidine hydrochloride on the activity of Method: The hydrolyzing activity of the enzyme was monitored using the denatured substrate the para nitro phenyl asetat. the activity of (pK) followed by the increased optical density (OD) of paranitrophenyl photometrically at 425 nm by using of UV-Vis spectrophotometry.samples is incubating in the pH 7.4 at 40 °C for 30 min .The concentration of enzyme is 0.04mg/ml and 1,2and 3M of GdHCl concentration.and using the sodium phosphate buffer.Results: In the low concentration of GdHCl activity of pK is increases but by increasing of concentration of GdHCl activity is reduced. [GdHCl] km VMAXnone 2.12 0.121M 1.46 0.162M 1.75 0.093M 2.32 0.085
Conclusions: The enzymatic and spectroscopic studies described in this paper, represent useful information for more understanding of the connection between structural changes and activity of pK . The change of pK structure can be study by Fluorescence Spectroscopy

Keywords: Proteinase K, activity, paranitrophenyl, spectrophotometry.
Title:
Design and Construction of a Monomeric Geobacillus Maltogenic Amylase by Insertion of Specific Linker between Domains N and A

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Abstract:

Introduction: Maltogenic amylase (MAase) is one of the most important enzymes in modern bakery industry and in contrast to other α-amylases acts in dimeric form and prefers cyclodextrins (CDs) to starch or pullulan as substrate. The under study MAase isolated from a locally thermophilic strain of Geobacillus species. It is in homodimer (chains A and B) form and each monomer consists of three domains including domains N, A and C. The active sites form as a domain swapped homodimeric structure between domains A and N. To designing a monomeric variant, two separated parts of the enzyme (A and C domains of chain A and N domain of chain B or vice versa) must be connected to each other by a specific linker. The desired linker here connects the A and N domains to each other.

Method: For linker designing, first, a few fragments were selected based on structural criteria from the Protein Data Bank. Next, recombinant proteins were modeled by MODELLER software. After that, Molecular Dynamics simulations and docking were performed for all the structures to help out screening of more relevant structures. Finally, the encoded gene of modified enzyme was synthesized and was inserted into pET-28a expression vector and over expressed in E. coli. The enzyme activity was determined by DNS method using B-CD.

Results: Molecular docking studies showed an acceptable affinity between enzyme complex and its substrates. Determination of enzyme activity showed that, as well as native monomeric enzyme form is active. Further experiments including purification and characterization of the protein are currently under way.

Keywords: Maltogenic amylases, Cyclodextrins, Linker design, Protein design.
Title: Inducing apoptosis and differentiation by chromene family derivatives in leukemic cell lines

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Abstract: Introduction
Leukemia is a particular type of cancer that manifested by the failure of cell death, or inability of hematopoietic cells. Chronic myelogenous leukemia (CML) is the most studied type of this cancer. In recent years many conventional methods were examined for treatment of human leukemia and new compounds were investigated to find out agents which has less side effects and more anti-tumor activity. Among different agents we can refer to chromene family derivatives. Pyranochromene derivatives are heterocyclic compounds with potent anti-tumoral activities.

Method
In our research leukemic cell lines were cultured in the presence of various concentration of the drug. Cell viability was determined using MTT assay. Also, we used NBT test for finding out the extent of differentiation cells. For morphological study of apoptotic cells, after staining the cells with Acridine orange/ethidium bromide (Ao/EtBr) we used fluorescence microscopy. Also, apoptosis was detected by DNA fragmentation assay.

Results
MTT assay was revealed that after treating cells with various concentration of drug for 24-72 h, cells viability was decreased. NBT test was showed that the inhibition of proliferation is associated with differentiation and morphological study of apoptotic cells revealed that apoptosis occurred after differentiation of the cells. Also, further confirmation of apoptosis came from the DNA fragmentation assay.

Conclusion
So we can say pyranochromene derivatives induce differentiation and apoptosis in leukemic cell line.

Keywords: Leukemia, CML, Chromene, Apoptosis
Title: Methylenetetrahydrofolate Reductase Genotype Combinations (MTHFR C677T and A1298C) in Women with Recurrent Pregnancy Loss of Northwestern Iran: A Comparison with a Healthy Control Group.

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Abstract: Introduction: The prevalence of the two common polymorphisms MTHFR C677T and A1298C of the 5, 10-methylenetetrahydrofolate reductase gene highly increases the risk of thrombophilic diseases such as recurrent pregnancy loss and deep venous thrombosis. Therefore, the current study was designed to determine of MTHFR C677T and A1298C polymorphisms and their relationship with recurrent pregnancy loss (RPL). Method: A total of 400 women were included in this study, 200 women with two or more consecutive pregnancy outcome as a case group and 200 healthy women as a control group. Genotyping of the 677CT and 1298AC polymorphisms in the 5, 10-methylenetetrahydrofolate reductase gene were carried out by the use of ARMS-PCR and PCR-RFLP techniques, respectively. Results: Our results showed that the frequency of the combined genotype MTHFR 677CC/1298AC among women with RPL was higher than the normal population. However, the differences were not statistically significant between two groups. Combinative genotypes of MTHFR 677CC/1298CC, 677TT/1298AA and 677CT/1298AA had a higher prevalence rate in RPL patients, compared to healthy women. While the prevalence of the 677TT/1298AA genotypes in case group was lower than the control group, the 677TT/1298CC, 677TT/1298AC and 677CT/1298CC genotypes in both populations of healthy controls and RPL women were not observed. Conclusions: Our study demonstrated the existence of an association between MTHFR C677T and A1298C polymorphisms in patients with RPL in northwest of Iran. Our results also showed that in individuals with the homozygous TT genotype of the C677T polymorphism and the homozygous CC genotype of the polymorphism A1298C has a further increased risk of developing RPL. Not observation of the 677TT/1298CC, 677TT/1298AC and 677CT/1298CC genotypes in the study of healthy and RLP populations strengthened this hypothesis the decreased viability among individuals carrying the 677CT/1298CC, 677TT/1298AC and 677TT/1298CC genotypes.

Keywords: 5, 10-methylenetetrahydrofolate reductase (MTHFR), Recurrent Pregnancy Loss (RPL), polymorphism, genotype.
Title: Production of bioethanol from wastewater sludge of the anaerobic digester of Isfahan's North wastewater treatment plant

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Abstract: Reutilization of wastewater sludge as a raw material in order to produce value added products is known as a new solution for sludge management since sludge contains a wide range of nutrients. The possibility of production of bioethanol, as a value added product, from anaerobic sludge was the main goal of this study. The sludge was obtained from the anaerobic digester of North wastewater treatment plant, Isfahan, Iran. The anaerobic treated sludge is a difficult substrate for biological conversion. In order to release the nutrients which exist in the sludge, acid pretreatment was conducted on the wet and dried sludge. To identify the performance of the treatment, Saccharomyces cervisiae was cultivated on both treated and untreated sludge. As it was expected, the highest and the lowest viable cell counts were related to the wet and dried treated sludge respectively. The untreated sludge did not show a good performance as a result of unavailability and non-degradability of organic materials to the yeast. However, further studies showed that the addition of glucose as a source of carbon can improve the growth performance of the yeast on the sludge. Besides, it was concluded that some parameters such as sludge solids concentration, sludge nature, initial inoculum, medium composition and pH can influence the growth performance as well.

Keywords: Sludge management, Value added products, Bioethanol, Pretreatment
Title:
New water molds for mycoflora of IRAN

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Abstract:
In a survey on water molds of different regions of Mazandaran, some water samples subjected to baiting with sterilized hemp and sesame seeds. Isolated fungi purified after culture on agar plates. For zoosporangia and oogonia production, five milimeters agar disks containing fresh mycelium transfered to petri plates filled with distilled water. After zoosporangia and oogonia production the microscopic characteristics were studied and after their coincidence with valid identification key(T. W. Johnson et al. 2002), the below species and genus were distinguished:

1-Achlya recurva; Sporangia clavate or fusiform, renewed sympodially. Spores monomorphic; discharge and behavior achlyoid, occasionally aplanoid; primary spore cysts 9-12 µm in diameter. Gemmae rare. Oogonia lateral, rarely terminal, very rarely intercalary; spherical, 45-86 µm in diameter. Oogonial wall unpitted; truncate and thin-walled at the apex. Oospores eccentric; spherical; 4-9 per oogonium, 16-33 µm in diameter. Antheridial branches androgynous, rarely monoloinous, very rarely dilinious, persisting. 2-The genus Dictyuchus; spore monomorphic, encysting within the sporangium and subsequently emerging individually from the cysts as reniform planonts, leaving the empty sporangium net-like; sporangium wall remaining intact and cysts being angular, or deliquescing, with the adhering cysts becoming rounded. cysts in more than single row in sporangium but occasionally in a single row. the sporangium diameter are 55-830 µm. The genus Dictyuchus sp. and the Achlya recurva are new records for mycoflora of Iran.

Keywords: Water molds, Oogonia, Zoosporangia,Mazandaran.
Title:
Analysis of the binding interaction of some natural and synthetic curcuminoids with hen egg white lysozyme

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Abstract:
Introduction:
Curcuminoids are natural polyphenolic coloring compounds in the rhizomes of Curcuma longa commonly known as turmeric. The major curcuminoids are curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcuminoids have significant therapeutic potentials as antioxidant, anticarcinogen, anti-mutagenic, and anti-inflammatory. Lysozyme is an antimicrobial proteinase that has ability to lyse the cell walls of bacteria. Lysozyme has different physiological and pharmaceutical functions, so studies on the interaction of drugs and bioactive compounds with lysozyme are important for elucidation the therapeutic effectiveness of drugs. In this study, we aimed to investigate the interaction of bisdemethoxycurcumin (BDMC) as a natural curcuminoid, diacetylcurcumin (DAC) and diacetylbisdemethoxycurcumin (DABC) as novel synthetic curcuminoids with chicken egg white lysozyme.

Method:
This study was carried out using different techniques including steady-state fluorescence, synchronous fluorescence, three-dimensional fluorescence, UV-vis absorption, fluorescence resonance energy transfer, and molecular docking.

Results:
The fluorescence experiments revealed that addition of curcuminoids quenched the intrinsic fluorescence of lysozyme by formation of a non-fluorescent complex (static quenching). The number of substantive binding sites and the binding constants were calculated by relevant fluorescence quenching data. Based on the Förster's theory, distance between the donor (lysozyme) and acceptor (curcumin) as well as the critical energy transfer distance has also been calculated. The molecular docking studies revealed that specific interactions were observed with the Trp-62 and Trp-63 residues.

Conclusions:
Our results showed the moderate binding affinities toward interactions between natural and synthetic curcuminoids with lysozyme. The comparison among the binding affinities revealed that phenolic hydroxyl groups and methoxy groups have significant role in the binding interactions.

Keywords: Lysozyme, Curcuminoids, Fluorescence Spectroscopy, Molecular Docking
Title:
Biological method for selenium nanoparticles synthesis assisted by \( \alpha \)-amylase enzyme from bacillus methylotrophicus

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Abstract:
Introduction: Metal nanoparticles have different applications in chemistry, biomedical and material sciences. One of the requirements for advancement of nanotechnology is the development of reliable experimental protocols for the synthesis of nanomaterials over a range of biological compositions, sizes and high monodispersity. The secreted proteins/enzymes and reducing agents such as amino acids, peptides and organic acids in biological entities were found to be responsible for nanoparticle production. Selenium nanoparticles have the effect against UV-induced DNA damage activities and have the applications in medical diagnostics.

Methods: Formation of selenium nanoparticles was investigated by using of the purified enzyme from Bacillus Methylotrophicus followed by adding aqueous concentrations of Na2SeO3 solution (0.05 to 10 mM) with different mole ratios (1:1, 1:2, 1:4, 1:5 and 1:10). The prepared mixtures were incubated at different temperatures (50 °C-70 °C) and the light red color after 24 hour was observed. UV-Vis spectra of the mixtures of nanoparticles were recorded in the range of 300–700 nm. Other characteristics of produced nanoparticles such as average particle size and FTIR were also determined.

Results: Biosynthesis of SeNPs occurred at 50 and 70°C in various range concentrations of Na2SeO3 with ratios of 1:2, 1:4 and 1:10. Optimization of selenium nanoparticle synthesis was also carried out. The average particle sizes of nanoparticle analyzed.

Conclusions: In the present study, Selenium nanoparticles were synthesized by pure \( \alpha \)-amyrase. Results showed that glucose accelerated the nanoparticle synthesis.

Keywords: Selenium nanoparticle, Enzyme biosynthesis, Bacillus Methylotrophicus, Alpha amyrase
Title:
Molecular simulation and docking study of interaction between Fisetin with human serum albumin

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Abstract:
Human serum albumin is the most abundant protein in human blood plasma. The reference range for albumin concentrations in blood is 3.4 to 5.4 g/dl. Albumin transports hormones, fattyacids, and other compounds. Flavonoids are a class of secondary plant phenolics with significant antioxidant properties which have been suggested to exert anumber of beneficial actions on human health, such as anti-inflammatory,anti-cancer and anti-tumor activities. In the human diet, they are most concentrated in fruits, vegetables, wines, teas and coca.Fisetin is a famous flavonoid. The binding of Fisetin with human serum albumin (HAS) has been studied at different pH using UV-Vis,FTIR, CD and fluorescence spectroscopic techniques. The binding constants were found to increase with the rise in PH of the media. The negative $\Delta H^0$(kJ.mol$^{-1}$) and positive $\Delta S^0$ (J.mol$^{-1}$.K$^{-1}$) indicate that fisetin bind to HSA via electrostatic interactions with an initial hydrophobic association that result in a positive $\Delta S^0$. In presence of potassium chloride (KCL) the binding constant were found to be decrease. In the present study, the interaction of Fisetin with human serum albumin (HAS) has been characterized by molecular simulation and molecular docking calculations. The results of docking suggested the best active site of HSA for this Flavonoid and molecular simulation determined the time evolution of protein-flavonoid complex.

Keywords: Fisetin, docking, molecular simulation, human serum albumin
Title:
The Study Of STR In E- Cadherin Gene: A New Era Of Breast Cancer Research

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Abstract:
Introduction: Breast cancer is the most common type of cancer and the second leading cause of cancer-related death among women word wide. one basic characteristic of cancer cells is that they adhere poorly to each other. cell-cell adhesion is mediated by a variety of membrane proteins such as cadherins. E-cadherin is the predominant cadherin family member that is expressed by all epithelial cells. The E-cadherin gene (CDH1) is located on 16q22.1 and produces a 120 kd protein. Loss of function of E-cadherin correlates with increased invasion and metastasis in breast cancer cells. Bioinformatic studies show a region with CTTT repeats in intron 2 of CDH1. To date there has been no study on this STR. The aim of this study is to analyze the CTTT repeats in intron 2 of CDH1 gene.

Method: After designing primers, Genomic DNA was extracted from the blood of 100 patients and 100 control, then desired fragments was amplified by PCR technique. The PCR products were analyzed by polyacrylamide gel.

Results: So far we have observed alleles with different number of CTTT repeats.

Conclusion: Characterizing the number of CTTT repeats in intron 2 of CDH1 and finding its relationship with breast cancer can help us in diagnosing people susceptible to breast cancer and also can have therapeutic purposes.

Keywords: STR, E-cadherin, Breast cancer, metastasis
Title:
The investigation of Isocitrate dehydrogenase (IDH) expression change in human brain Oligodendroglioma tumor

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Abstract:
Isocitrate dehydrogenase (IDH) catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate and reduces NAD (P) to NAD (P)H. IDH1 and IDH2 are NADP dependent. IDH1 mutation is frequent in diffuse Gliomas such as Oligodendrogliomas. Oligodendroglioma tumors continue to receive much attention because of their relative sensitivity to chemotherapy. In an attempt to get an understanding of molecular diagnosis of Oligodendroglioma tumors. We extracted proteins of tumor and normal brain tissues and then evaluated the protein purity by Bradford test and Spectrophotometry method. In this study, we separated proteins by 2DG Electrophoresis method and the spots were then analyzed and compared using statistical data and specific software, after providing 3D images of spots alteration. Spots were identified by PI, molecular weights and data banks. Oligodendroglioma with a mutant IDH1 had noteworthy enhanced expression of enzymes controlling aerobic glycolysis and detoxification and anti-apoptosis proteins. To date, all IDH1 mutations have been identified at the Arg132 codon. Mutations in IDH2 have been identified at the Arg140 codon. Comparative proteomics analysis might thus be suitable to identify proteome alterations associated with a well-defined mutation.

Keywords: IDH, Proteomics, Oligodendroglioma and 2D-DIGE
Title:
Expression profile of EYA1 gene in gastric cancer tissues

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Abstract:

Introduction: As the second most frequent cause of cancer death, gastric cancer is a common disease worldwide, with a frequency that varies greatly across different geographic locations. In Iran, especially in northwest, gastric cancer is the major cause of cancer-related mortality, especially in men. Most human cancers show heterogeneity in their genetic profiles. EYA1 gene encodes a transcriptional co-activator protein which is required for normal development of different tissues. Furthermore, previous studies demonstrate that EYA1 is overexpressed in several tumor types such as Wilms' and neuroblastic tumors. The aim of this study was to evaluate the expression profile of EYA1 gene in gastric carcinoma.

Method: 30 paired fresh frozen tumoral and non-tumoral gastric tissue samples were examined. RNA extraction and cDNA synthesis were performed according to manufacturer's instructions. Then conventional and quantitative real-time RT-PCR was done for evaluation of the expression of EYA1 gene.

Results: Our results showed that the expression of EYA1 gene was heterogeneous in gastric specimens. Furthermore, there was no significant alteration in EYA1 expression between tumoral compared to non-tumoral tissues, different tumor types and grades. Conclusions: Collectively, our results call for further investigation to precisely define the role of EYA1 in normal and pathological conditions of major human organs including stomach.

Keywords: Key words: Gastric cancer, EYA1 gene, quantitative real-time RT-PCR
Title: Determination of the sensitivity and specificity of PCR by using different gene primers to detect Helicobacter pylori in gastric biopsy specimens

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Abstract: Introduction Almost half of the world's population undergoes from the Helicobacter pylori (H. pylori) infection. This microorganism is accepted as the most important agent of gastritis and as a risk factor for peptic ulcer disease and gastric adenocarcinoma. several invasive and noninvasive tests are currently available for diagnosis of H. pylori. Methods based on molecular biology are considered highly specific and sensitive tests, and many PCR-based assays have been developed to detect H. pylori DNA in gastric biopsies. However, this technique is able to detects specific fragments but not viable bacteria, and its sensitivity also depends on several factors. The aim of this study is to determine the sensitivity and specificity of PCR primers to diagnose Helicobacter pylori infection. Method One hundred H. pylori strains will isolate from patients with different gastrointestinal disease who referred to Tabriz Emam Reza hospital. Any sample positive on histological examination as well as rapid urease test was considered as the gold standard for determination of the sensitivity and specificity of the PCR methods. ResultsPreliminary results are provided and the final results will be presented in Congress. Genomic DNAs were extracted from all strains. 20 sample was examined by different PCRs. ConclusionsDiscussion after obtaining the final results will be presented in Congress.

Keywords: Helicobacter pylori, Gastric Disease, PCR, Histological examination, Rapid urease test
Title:
Activity studies of native human tyrosinase and its two mutational variants in HEK-293 cells

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Abstract:
Skin, hair and eye colors are due to a pigment called melanin. It protects them from suninduced damage and synthesized by specialized cells called melanocytes. Melanins are polymers of phenolic compounds. The more general classification of such compounds, including all their types in Pro- and Eukaryota, contains three main types called eumelanins, pheomelanins and allomelanins.

In the sequential pathway of melanin formation, tyrosinase is the rate-limiting enzyme that catalyzes tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and oxidizes DOPA to dopaquinone. It also has been shown that melanin biosynthesis pathway can be feasibly induced using recombinant tyrosinase. Herein, our goal was to evaluate the activity of native tyrosinase and its two mutational variants in HEK-293 cell line.

Two mutants of human tyrosinase enzyme have been previously constructed in our lab anticipated to possess higher catalytic activity in HEK-293 cells in comparison with the native protein. Native enzyme and the mutational variants have already been cloned in pET-28b(+) for evaluation of protein expression in Escherichia coli BL21. In this approach, coding sequences of native and mutant proteins have been subcloned into pcDNA3.1(+) expression vector followed by subsequent transfection of HEK-293 cells with three constructed pcDNA3.1(+) -hTyr vectors. Activity studies of native tyrosinase and two mutational variants have been performed and will be presented in congress.

Keywords: Subcloning, Tyrosinase, Mutational variants, HEK-293 cell line, Activity studies
Title:
Co-expression of Artemin with Firefly Luciferase in E. coli for Real-time Monitoring of Preventing Oxidative Stress by Artemin

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Abstract:
Artemin is a major protein of Artemia that protects its encysted embryos from environmental stress. We have previously cloned artemin cDNA from encysted embryos of Artemia urmiana. We found that artemin acts as an efficient molecular chaperone in vitro and confers thermotolerance to E. coli cells expressing artemin. In this study, we have decided to investigate the effect of artemin on E. coli under oxidative stress. Effects of H2O2 treatments on cell growth were determined, and observed that artemin expression in E. coli confers oxidative resistance to these cells at lethal concentrations of H2O2. Co-transformation of E. coli BL21(DE3) was also performed with two expression vectors containing artemin and firefly luciferase (Luc) for in vivo studies of oxidation induced aggregation of this test substrate and the protective role of artemin. Luc activity was monitored under oxidative stress as an intracellular reporter. The transformants were subjected to oxidative stress treatment at different concentrations of H2O2. Similar to cell growth, residual activity of Luc in induced cultures over-expressing artemin was significantly higher than non-expressed artemin cells (control). In summary, our in vivo experiments suggest that artemin protects cells from oxidants, leading us to conclude that we have found a protein that plays a significant protective role under oxidative stress in E. coli cells. Like to heat shock assay, Luc can provide a suitable test substrate for real-time monitoring of preventing oxidative stress as a rapid and sensitive in vivo assay.

Keywords: Artemin, Luciferase reporter, Co-expression, in vivo assay
Title:
Wnt molecules role in viability, adhesion and migration of SKOV3 human ovarian cancer cell line

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Abstract:
Wnts are secreted acetylated glycoproteins that can act as autocrine or short-range signaling molecules and long-range morphogens which play important roles in development and cancer. Small molecule IWP-2 is an inhibitor of Porcupine activity which is a membrane-bound acetyltransferase that is essential to the production and biological function of Wnt proteins. This study sought to determine the role of Wnts molecules in viability, proliferation, migration and adhesion of human ovarian cancer cell line SKOV3 by using IWP-2. To this end, cells were treated with IWP-2 (2, 5, 7.5 and 10 µM) for 48 h without serum. Cell viability and/or proliferation were determined by using MTT assay. There was 49, 59 and 62% decreased cell viability compared to untreated cells (control) with 5, 7.5 and 10 µM IWP-2 for 48 h, respectively (P<0.01). Wound healing assay showed increased cell migration in cells treated with IWP-2 (224% versus 100% in control, P<0.01). Moreover, adhesion assay demonstrated decreased cell adhesion by 48% compared to control 30 min after seeding (P<0.01). Our data suggests that Wnt molecules play important role in viability, adhesion and migration of human ovarian cancer cell and may be implicated in ovarian cancer progression.

Keywords: Ovarian cancer, Wnt proteins, IWP-2, human ovarian cancer cell line SKOV3
Title:
Identification of Glutamate decarboxylase gene in Lactobacillus delbrueckii subspecies bulgaricus

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Abstract:
Introduction: Glutamate decarboxylase (GAD) is a pyridoxal 5′-phosphate (PLP)-dependent enzyme, which catalyses the irreversible decarboxylation of L-glutamate to Gamma-aminobutyric acid. Gamma-aminobutyric acid (GABA) is a non-protein amino acid known to be a major inhibitory neurotransmitter in the mammalian brain tissues. GABA has several physiological functions such as neurotransmission, hypotensive activity, as well as tranquilizing effects, particularly with regard to insomnia, depression and autonomic disorders, prevents diabetic, it may be used for treatment of chronic alcohol-related symptoms and sleeplessness. The aim of this study is to identify glutamatate decarboxylase gene sequences in lactobacillus delbrueckii subspecies bulgaricus. Methodes: Some bacteria such as Lactobacillus strains that were isolated from yogurt could produce GABA. In the current study we analysed phylogenetic genus of Lactobacillus delbrueckii subspecies bulgaricus based on the 16S rDNA sequence and biochemical studies. Then primers were designed from highly conserved regions of GAD and were used to amplify genes for GABA-synthesizing enzymes. The PCR product was purified from gel and was sequenced. Result: The results of this study suggested that this strain could produce GABA. Conclusion: Lactobacillus delbrueckii subspecies bulgaricus could produce GABA and it may be used as a bioreactor for producing GABA naturally. It also may use as a probiotic producing GABA.

Keywords: Glutamate Decarboxylase, Gamma-aminobutyric acid, Lactobacillus delbrueckii subspecies bulgaricus, PCR
Title: Study of the immobilization of bacteriorhodopsin by using the spin coating method in protein nano-memory

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Abstract: Introduction: Protein Memories are the novel technology that uses the proteins and biological Molecules latent abilities in order to electronic Processing of data. In Bioelectric, many researches have been done on Bacteriorhodopsin as a sample protein. The optical and physical properties of bacteriorhodopsin were used to produce optical nano-memories. In addition, photochromic properties of Bacteriorhodopsin are used most in molecular electronic devices. In this present study, the spectrophotometric data was analyzed to determine the optimal concentration of film containing BR. Also we studied immobilization of BR on Polycarbonate layer of the CD by the physical method.

Method: Polyvinyl alcohol and Gelatin in different weight/volume ratio were prepared to immobilize protein. Also Bactriorhodopsin protein in different concentration was used.

Results: The optimum concentration of polymer films after immobilization of BR was analyzed. This solution was injected on polycarbonate layer and then immobilized by Spin-Coater machine that is a physical method. AFM imaging was also performed. This study shows that these BR immobilized films with a good optical activity, with nanometre thickness range, with high homogeneity and optical transparency are good method for producing protein memories.

Keywords: Bacteriorhodopsin, polycarbonate layer, Protein Memories, Spin coating
Title:
Computational Study on Single-Walled Carbon Nanotube and Herceptin Drug in gas and water phase

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Abstract:
Introduction:Recently, the majority of the investigations into computational chemistry has been focused on the examination of nano drugs in various phases and for example the interaction of anticancer drug with carbon nanotubes (CNTs). The most likely tendency is toward the interaction between anticancer drug and single-walled carbon nanotubes(SWCNTs). In this investigation, the interaction of Heceptin (Trastuzumab), a common form of anticancer drug in case of breast cancer with open-end of SWCNT, are examined. We have examined loops of Herceptin and its linkage to carbon nanotube in two phases. Gas phase and water phase. So we calculate interaction of Heceptin with SWCNT, utilizing these force fields. The goal is simulation of linkage process of Herceptin loops with SWCNTs and reach to calculation results.
Method: In this study we have employed semi-empirical and molecular mechanic methods. The calculations have been done with the Hyper Chem 8 program. We have investigated our study in different temperatures(300,302,304 and306 k) for calculating in Monte Carlo (MC),Molecular Dynamic(MD) and Langevin Dynamic(LD) all according to molecular mechanic theory. Then we have compared in two phases. In case of semi-empirical, we have computed total energy ,binding energy,heat of formation ,core-core interaction ,electronic energy and gradient of the loop-nanotube in both gas and water phases. Results: Finally we compared our data with experimental data reported in articles and we reached to some similar results. So we found more stability of compound in water phase. Conclusions: After all data must be used for nano drug delivery so before doing the job we strongly suggest: Calculating with different methods and technology of simulations. Using different solvents. Study of other carriers for drug delivery (Q-dots, Nano shells, Nano liposomes,..)

Keywords: Keywords :single-walled carbon nano tubes(SWCNTs), Herceptin ,semi-empirical method and molecular mechanics method
Title:
Prediction of Epitopes of Bacillus cereus Hemolysin

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Abstract:
Introduction: Bacillus cereus, which is best known as the cause of two distinct food poisoning syndromes, has also been implicated in a wide variety of illnesses. The virulence factor of this bacterium remain ill defined partly because a large number of proteins, like hemolysin. Hemolysins are exotoxins produced by bacteria that cause lysis of red blood cell by damaging their cell membrane. in this study, we try to design new vaccine against Bacillus cereus hemolysin enzyme for diarrhea therapeutic procedures. Method: The identification and characterization of B-cell epitopes play an important role in vaccine design, immunodiagnostic test, and antibody production. Therefore, computational tools for reliably predicting linear B-cell epitopes in proteins are highly desirable. Here we use ElliPro server is a web based tool that aims to predict immunogenic regions in either a protein three-dimensional structure or a linear sequence. The ElliPro output consist of the immunogenicity and corresponding probability scores are computed by ElliPro for each surface residue. Results: As it is shown in the result, the first peptide with highest score is the best option for epitope prediction and we can use this peptide for vaccine design against Bacillus cereus hemolysin enzyme. Conclusion: to reduce bacterial drug resistance and faster effect of vaccine in comparison to oral drug vaccine design against this bacterium is recommended.

Keywords: Hemolysin, Bacillus cereus, ElliPro server, Epitope prediction
Title:
A Survey of the fatty acid content of human oral squamous cell carcinoma

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Abstract:
Introduction: Oral squamous cell carcinoma appear to have an altered lipid metabolism. The aim of this study was to investigate differences in tissue fat composition between malignant and adjacent normal squamous tissue.

Method: Normal-appearing and malignant squamous tissue were collected from 25 patients with oral squamous cell carcinoma. The fatty acid composition in the obtained tissue was determined by gas liquid chromatography.

Results: In the squamous cell carcinoma tissue, the level of stearic acid (18:0; P<0.001) was higher, and the levels of oleic acid (18:1n-9; P<0.001) and linoleic acid (18:2n 6; P<0.001) were lower than that in the normal-appearing squamous tissue. Overall, squamous cell carcinoma had a significant reduction in the total n-6 polyunsaturated fatty acid (-13.1%; P<0.001).

Conclusions: The change in the fatty acid composition may be regarded as an indicator of altered lipid metabolism occurring in vivo during squamous cell carcinogenesis.

Keywords: fatty acids; squamous tissue, squamous cell carcinoma
Title: 
Evaluation of IgE against 20 Common Allergens by Immunoblotting Method in Atopic Dermatitis Patients

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Abstract: 
Introduction: Stimulating the immune system by Exposure to various allergens to produce specific IgE has a significant role in the pathogenesis of atopic dermatitis. Identifying disease-causing allergens and prevention of exposure to those allergens and immunotherapy will play important role in the treatment of atopic disease. Purpose of this study was to determine the common allergens of East Azerbaijan in patients with atopic dermatitis. Materials and methods: In this study serum levels of total and specific IgE were measured by Western blot against 20 common allergens in 150 patients (mean age=29.02±14.79 years, 77 patients (51.3%) = male and 73 patients (48.7%) = female) with atopic dermatitis attending to dermatology clinics and asthma and allergy clinics from 2010 to 2011. Control group consisted of individuals who had been diagnosed healthy. Results: In the patients that were included in this study (90%) total IgE levels (mean=227.51±103 IU/ml) were higher than healthy people and 136 patients (90.6%) had specific IgE for at least one allergen. Most abundant allergens respectively related to: cultivated rye (48.6%), Timothy grass (42.9%), house dust mites (22.7%), Cat (16.7%), horse (10%), birch (11.3%), Potato (11.3%), dog (16.7%), egg white (8.7%), cow milk (8.7%). sagebrush, wheat, rice, fish, soybeans, nuts and apples had little frequency. Carrot was not detected in none of the cases. Conclusion: Identifying the abundant allergens such as Cultivated rye, Timothy grass, House dust mite, birch, Cat, Horse, Potato, Dog, Egg white, Cow milk is order to advise patients to avoid them or do immunotherapy and desensitization is useful in this area.

Keywords: atopic dermatitis, allergen, immunoblotting, IgE,
Title:
Investigation of Blood Melatonin Levels of Obese and Nonobese Subjects

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Abstract:
Aim: The aim of the study was to investigate blood melatonin levels and insulin resistance and correlations between them in obese and nonobese subjects. Materials and Methods: The study was performed on 33 (16M, 17F) obese subjects aged 18-65 years and 33 (15M, 18F) normal weight healthy controls aged 18-65 years. In both groups melatonin level was determined by ELISA method and insulin resistance was calculated using a formula. Findings: There were no significant differences between melatonin levels of the obese (115.52 ± 9.0 ng/mL) and nonobese (129.47 ± 9.0 ng/mL) subjects. Also, there were no correlation between melatonin and insulin resistance levels in both groups. Conclusion: Our results show that melatonin does not play a significant role in the pathogenesis of obesity.

Keywords: Obesity, Melatonin, Insulin Resistance
Title:
Sub-MIC Concentrations of Allicin (an active component of garlic) Inhibits Biofilm Formation by Pseudomonas aeruginosa

Authors:
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Abstract:
Background &Objective: Pseudomonas aeruginosa is an important opportunistic pathogen, causing a wide range of infections. Biofilm formation contributes to pathogenesis of Pseudomonas aeruginosa lung infections in patients with cystic fibrosis. Cells in the biofilm show higher degree of resistance to antimicrobial therapy and host immune responses compared with planktonic cells. So inhibition of biofilm formation could help the body’s immune system to combat the bacteria and improve the clinical outcomes for antimicrobial therapy.

Material & Methods: This study was carried out using a P. aeruginosa 8821M. Allicin was purified using semi preparative HPLC procedure. MIC of allicin was determined by microdilution method using serial dilutions of aqueous allicin solution (4-1024 µg/ml) in LB broth. Biofilm inhibition was assayed using Microtiter plate method in the presence of sub-MIC concentrations (4µg-128µg) of allicin. The plates were incubated for 18 hours at 37 °C. Bacterial biofilms were stained with 0.2% safranin. Dye was solubilized using alcohol-Aceton as solvent and the optical density (OD) was measured at 492 nm wavelength. The extent of biofilm formation was determined (OD of sample well/OD of control well*100). Each assay was performed in triplicate and repeated two times.

Results: The allicin MIC was 256 µg/ml for P. aeruginosa. The results indicated that allicin at concentrations of 16 and 32µg/ml significantly diminished biofilm formation (P<0.05). This concentration did not have significant influence on bacterial growth rate.

Conclusion: The results showed that allicin can inhibit the biofilm formation by P. aeruginosa.

Keywords: Pseudomonas aeruginosa, Biofilm inhibition, Allicin
Title:
cDNA synthesis of the RNA of SRI gene in leukemia patient samples

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Abstract:

Introduction: Multidrug resistance (MDR) is a major factor of treatment failure in various leukemias. The increase of expression of some genes such as SRI, that codes a 22KDa calcium-binding protein; namely Sorcin, in cancer cells may increase the possibility of MDR. Sorcin plays a fundamental role in emerging intra-cellular calcium homeostasis and causing drug resistance in various cancers through regulating the expression and functions of some apoptotic factors and P-gp. The study of the expression changes of Sorcin in cancer cells may help improving treatment of MDR. The main objective of this research is RNA extraction and cDNA synthesis from de novo acute lymphoblastic leukemia (ALL) cases for specific studies.

Methods: Bone marrow samples were taken from 30 ALL new case patients. RNA extraction from mononuclear cells was carried out by using RNeasy Mini kit. In order to provide total cDNA, Fermentas kit was used. Specific primers for Sorcin were designed by AllelID 7.7 and Oligo 7 programs. The specificity of primers was evaluated by PCR assay.

Result: The counting of the cells taken from bone marrow samples indicated that the number of the required cells was sufficient. RNA density measurement by bio-photometer apparatus indicated the net percentage of notable extracted RNA. Additionally, the specific bands of products were observed followed by polymerase chain reaction and Gel electrophoresis, confirming the precision of primers and the total cDNA.

Conclusion: Through Real-time PCR method, it is possible to measure the expression level of genes such as Sorcin in patient samples by providing total cDNA and designing specific primers.

Keywords: Multidrug resistance; Leukemia; Gene expression; cDNA; Primer.
Title: Isolation and differentiation of mesenchymal stem cells from adult rat bone marrow

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Abstract: introduction: The first non-hematopoietic mesenchymal stem cells (MSCs) were discovered by Friedenstein and he described clonal, plastic adherent cells from bone marrow capable of differentiation. MSCs have been studied in regenerative medicine because of their unique immunologic characteristics. MSCs are multipotent cells which can give rise to mesenchymal and non-mesenchymal tissues in vitro and in vivo.

Materials and Methods: Rat bone marrow mesenchymal stem cells were isolated from bone marrow aspirates of 10-20 ml taken from the iliac crest of male rats. and cultured at 37 °C in humid air with 5% CO in DMEM containing 20% fetal bovine serum (FBS) (Gibco), penicillin (100 U/ml), streptomycin (100 mg/ml). The medium was changed to remove the nonadherent cells at 24 h after seeding, and every 3 days thereafter. Then the primary and the first passaged MSCs were exposed to different concentrations (3, 5 and 10 mM) of 5-azacytidine for 24 h on day 3 of culture. The experiment was repeated in three 24-well plates with 12 wells for each treatment.

Results: The dynamic changes in morphology and the growth properties were compared with the untreated cultures at indicated time points.

Conclusion: Rat MSCs (rMSCs) sharing the same morphological and functional characteristics as human MSCs can be successfully isolated from adult bone marrow without previous rat or bone marrow treatment. Therefore, rMSCs will be an important tool to study the in vivo behaviour and fate of this cell type after grafting in rat pathology models.

Keywords: Bone Marrow Stroma, Mesenchymal Stem Cells, Cell Differentiation
Title:
Study on the interaction of bisdemethoxycurcumin with human alpha1-acid glycoprotein

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Abstract:
Introduction: Turmeric which is derived from the powder rhizome of Curcuma longa, is used as a spice and food preservative. The yellow color of turmeric is mainly related to curcuminoid compounds including curcumin, demethoxycurcumin and bisdemethoxycurcumin. Curcuminoids have a wide spectrum of biological activities such as antifungal, antidiabetic, antioxidant, antiinflammatory, anticancer, antiallergic, antiprotozoal and antibacterial activities. Bisdemethoxycurcumin is the most active of the curcuminoids present in turmeric for modulation of MDR-1 gene. Human α1-acid glycoprotein (AGP) is the major acute phase protein, that is negatively charged at physiological pH and its concentration increases in response to systemic tissue injury, inflammation, and infection. AGP consists of a chain of 183 amino acids contains 40% carbohydrate by weight and has up to 16 sialic acid residues.

Method: In this study, spectroscopic techniques such as steady-state fluorescence, synchronous fluorescence, fluorescence resonance energy transfer (FRET), and molecular docking have been used.

Results: Experimental results revealed that some tryptophan residues are involved in the binding of bisdemethoxycurcumin to AGP. The binding parameters including number of binding sites and binding constant have been calculated based on the fluorescence quenching data. The synchronous fluorescence results showed that binding of bisdemethoxycurcumin causes some changes in the conformation of AGP.

Conclusions: Although plasma concentration of AGP is much lower than that of albumin, AGP can become the major drug binding macromolecule in plasma with significant clinical implications. Characterization of the binding of natural and synthetic bioactive compounds to AGP is valuable for further designing efficient drugs and guiding clinical therapy.

Keywords: Bisdemethoxycurcumin, human alpha1-acid glycoprotein, Ligand binding
Title:
Study of short tandem repeats length in Six1 gene promoter

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Abstract:
Introduction: Recently, Numerous studies have been conducted to catalogue human genetic variations and their association with many complex traits and important diseases. A short tandem repeat (STR) is a type of DNA polymorphism where short sequences of DNA are repeated. Several studies have suggested STRs as convenient markers that will help to improve diagnosis and risk assessment. The SIX1 gene is part of a group of similar genes known as the SIX gene family which encode transcription factors. Expression of the SIX1 protein is important for normal development but its overexpression in many adult tissues have been reported in several types of cancer such as breast cancer.

Method: Using bioinformatic studies, a region with CA sequence repeats in the promoter of Six1 was detected and the primers were designed by means of Oligo software. Genomic DNA was extracted from blood samples. The desired fragment of Six1 gene promoter was amplified by PCR technique. Finally, the length of the repetition sequence is evaluated by polyacrylamide gel and direct sequencing.

Results: Presence of polymorphisms in this region has been elucidated and 5 different alleles were detected up to now.

Conclusions: Polymorphisms in Six1 gene can be correlated with its expression. So in this study the number of CA repeats in the promoter region of this gene was investigated, also if we can elucidate its relationship with breast cancer, it can be used as a prognostic factor.

Keywords: Six1 gene, STR, promoter, Breast cancer.
Title:
Investigation of the intron insertion effect in episomal eukaryotic vector on IL-2 secretion

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Abstract:
Introduction: The aim of this study was to investigate the effect of Intron insertion on the secretion level of IL2 in Jurkat cell line. The rising rate of cancer-related diseases and mortality undergoes the need for new approaches to directly target and fight cancer. T-cells expressing chimeric receptors are able to discriminate between antigen expressing and normal cells, they are well suited to penetrate and destroy solid tumors compared to antibodies. Furthermore, it has been observed in literatures that in most cases introns can influence regulation of gene expression at different levels owing to their various regulatory elements.

Methods: Therefore we used a chimeric cassette consisting of; llama VHH, co-stimulating and signaling fragments and a suitable spacer (in PCDNA3.1 shuttle vector). A eukaryotic intron fragment from a ribosomal protein was inserted in the upstream of the vector’s promoter. Then it was proliferated in a host (DH5α), extracted and transformed into Jurkat cell line using electroporation method.

Future work: For investigation of IL2 expression in RNA level, RT-PCR will be performed. The function of these transformed cells will be studied by co culturing the Jurkat cell line with MCF7 (expressing MUC1 antigen) which induces the expression of IL2 in Jurkat cells.

Result: We expect to observe that the insertion of intron in the upstream of the CMV promoter causes an increase in IL2 production compared to the vector without intron.

Conclusion: Performing further studies on in vitro models could make it an effective method against cancers.

Keywords: Chimeric cassette, T-cell therapy, Intron insertion, IL2
Title: Computer-aided design new Alpha Glycosidase Inhibitors and comparison with acarbose

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Abstract:
Introduction: Alpha-glucosidase (EC 3.2.1.20; a-D-glucoside glucohydrolase) inhibitors are oral anti-diabetic drugs used for diabetes mellitus type 2 that work by preventing the digestion of carbohydrates. Alpha-glucosidase inhibitors may also be useful in patients with diabetes mellitus type 1; however, this use has not been officially approved by the Food and Drug Administration. Inhibitors of the alpha-glucosidases that are involved in the biosynthesis of N-linked oligosaccharide chains have been reported to have antitumor, antiviral, and apoptosis-inducing activities, and some have been used clinically. Acarbose also blocks pancreatic alpha-amylase in addition to inhibiting membrane-bound alpha-glucosidases.

Methods: New alpha-glucosidase inhibitors by means of a computer-aided drug design protocol involving homology modeling of the target protein and the screening with docking simulations under consideration of the effects of ligand solvation in the binding free energy function were designed. MD simulations were performed using GROMACS program (version 4.5.1) with the GROMOS force field.

Results: The structure of B. cereus and S. cerevisiae α-glucosidase were taken as template. Alpha-glucosidase inhibitors are saccharides that act as competitive inhibitors of enzymes. The binding mode of the acarbose with the active site residues provided important information of catalytic site. Furthermore, the free energies of binding (ΔGb) and inhibition constants (Ki) calculated by AutoDock. Validating the docking simulation protocol was also tested. MD simulations were performed to determine the stability of the homology modeled structure α-glucosidase and to prove the binding mechanism as shown in the docking results.

Keywords: Alpha-glucosidase inhibitors; Computer-aided drug design; docking; Acarbose; Gromacs
Title: Isolation and cloning and expression of the mouse metallothionein-3 in prokaryotic system

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Abstract: Metallothioneins (Mts) are a family of low molecular weight, cysteine rich and heavy metal binding proteins. These proteins have many functions, such as metal detoxification and antioxidation. Genomes of eukaryotes include multiple MT coding genes. Scientists classify metallothioneins in four groups: Mt1, Mt2, Mt3 and Mt4. Mt1 and Mt2 encode for ubiquitous proteins, while Mt3 and Mt4 evolved to accomplish specific roles in brain and epithelium, respectively. Mt3 have unique functions such as inhibition of neurotic sprouting and its expression is markedly reduced in Alzheimer's disease (AD) brain. Our RT PCR experiment demonstrated expression of mouse Mt3 mRNA in brain tissue during the postnatal period of 30 days. In this study we isolated, sequenced, cloned and expressed the mouse Mt3 cDNA in prokaryotic system. Mouse Mt3 is highly over expressed in the brain tissue. The total RNA extracted from the mouse brain tissue was purified and reverse-transcribed into cDNA using oligo(dT) primer. ORF of Mt3 gene was cloned in pET22b+ vector and transferred into E. coli Top10F and Bl21 strain. Clony PCR, nucleotide sequence and blast analysis revealed that Mt3 have cloned correctly. Protein expression of recombinant construct was analyzed by SDS-PAGE and next Western Blot. Cells transformed with recombinant plasmid can use for sequestration of heavy metal ions. In addition, more experiments are necessary for purification and treatment of purified protein with neuron cells in functional analysis aims.

Keywords: Metal absorption, Mouse Metallothionein3, Cloning, prokaryote.
Title:
Study of Allele frequency and heterozygosity rate of rs28370188 in human coagulation factor VIII gene

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Abstract:
Introduction: Hemophilia A is an X-linked recessive coagulation disorder because of deficiency of blood coagulation factor VIII. In view of the size and the complexity of the factor VIII gene and large numbers of mutations reported associated with hemophilia A, molecular diagnosis of the disease using polymorphic markers, located in the F8 gene and closed it, is favorable. Thus, analysis of informative markers in F8 gene region, is an important step in linkage analysis and molecular diagnosis of the disease in Iranian population.

Method: In this study by use of different bioinformatic tools, a single nucleotid polymorphic marker (rs 28370188) at 5’ region of the F8 gene were investigated. This marker is located 1850 bp upstream of exon 1 in promoter of the F8 gene. Thus genomic DNA was extracted from blood sample of 160 unrelated individuals and 10 non-hemophilic family. Tetra-primer ARMS-PCR was used for genotyping detection of C/T SNP. Primers were designed for rs28370188 using oligo software. Genome amplified by ARMS PCR and 2 allele specific amplicons separated by gel electrophoresis. Results: we have detected genotype of F8 gene C/T SNP in healthy cases by gel electrophoresis. After genotyping of marker, allele frequency and heterozygosity of the marker is under investigation.

Conclusion: Given the importance of the 5’ region of F8 gene and different mutations involved in the hemophilia A, introduction of informative molecular markers in this region and genotyping them could be useful to determine phase and heterozygosity and following, molecular diagnosis of the disease in Iranian population.

Keywords: hemophilia A, factor VIII gene, allele frequency, Iranian population
Title:
Structural Analyses of Non-specific Lipid Transfer Protein2 for Drug Delivery Systems: a Bioinformatics Study

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Abstract:

Introduction: Drugs used in medicinal therapies should be stable and protected against environmental conditions to increase their effectiveness. Lipid transfer proteins (nsLTP), a group of small and basic proteins, are most abundant in plant. Based on the molecular weight, LTPs have been classified into two groups: nsLTP1(9kDa) and nsLTP2 (7kDa). These highly stable proteins can protect drugs against oxidation or degradation. In this study, the primary and secondary structures of nsLTPs have been analyzed by bioinformatics servers. The structure of nsLTPs was predicted using M4T server and the 3D structure of rice-nsLTP2 was analyzed. Moreover, we studied the effects of acylation and mutagenesis on the structure, lipid binding, and transfer activity of nsLTPs. Methods: At first, the primary and secondary structures of nsLTPs were studied using the software that is available through the ExPASy World Wide Web server (www.expasy.org/). The 3D structures of the nsLTPs were modeled with the modeling M4T server (http://manaslu.aecom.yu.edu/M4T/) and Chimera 1.7rc software. Analysis and comparison of the structures were carried out using Swiss-PdbViewer ver3.7. Results: By comparing the primary sequences of nsLTP2s, it was revealed that there is at least 40% similarity between different nsLTPs. The hydrophobic cavities in several relatively small nsLTPs have perfect size for drug binding. Moreover, studies demonstrate that mutagenesis (such as F39A) and acylation of nsLTPs modifies the protein conformation to increase the exposed hydrophobic surface. Conclusion: The results may help us to develop manipulated proteins that being useful to drug delivery system.

Keywords: KEYWORDS: nsLTPs, Drug delivery, Homology modeling, Mutagenesis
Title: Effect of salt stress on some growth and physiological parameters of Lycopersicon peruvianum under In vitro culture

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Abstract: Salt stress is one of the most serious environmental factors limiting the productivity of crop plants. This is due to the fact that salinity affects most aspects of plant physiology, growth and development. Lycopersicon Peruvianum L. belongs to the family Solanaceae. It is an annual and herbaceous plant. In this study, the effect of NaCl (0, 60, 90, 120 mM) were investigated on Lycopersicon Peruvianum. Results showed that the fresh and dry weight decreased at concentration of 120 mM NaCl compared with concentration of 0, 60, 90. There was not significant difference among other salt concentrations. Chlorophyll and carotenoid content were also decreased with increasing of NaCl concentration. Prolin and sodium content increased with increasing of salinity compared to the control, but the potassium level was decreased, these result showed that sodium influx inhibited potassium uptake. It can be concluded that Lycopersicon peruvianum was able to survive in salin medium up to 120 mM NaCl which indicating that Lycopersicon Peruvianum is relatively salt tolerant.

Keywords: Lycopersicon peruvianum, Salt stress, In vitro culture
Title:
Effect of Sudlow site I drugs on human serum albumin esterase-like activity

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Abstract:
Introduction: Human albumin possesses remarkable promiscuous catalytic activities toward a broad range of organic molecules, including esters, amides, phosphates and benzisoxazoles. In particular, it has been previously reported that the hydrolysis rates of aspirin were greatly enhanced in the presence of HSA, confirming the esterase-like activity of HSA and contribution of site I, especially Lys199 was also proposed. “Esterase-like” activity (hydrolysis of p-nitrophenol esters pNPAc) by subdomain IIA has been also reported and site-directed mutagenesis studies have shown that Arg410 and Tyr411 are essential in this esterase activity. According to the above discrepancies, here, we set out to evaluate effect of some Sudlow site I drugs on the esterase activity of HAS as site marker in order to detailed study of drug binding sites.

Method: The kinetics of the pseudo-enzymatic hydrolysis of pNPAc was obtained in the absence and presence of drugs and binding site markers using UV-vis spectroscopy.

Results: The kinetics of the pseudo-enzymatic hydrolysis of pNPAc was obtained in the absence and presence of drugs and binding site markers. Indomethacin (as site I marker) and osthole inhibited competitively the HSA-catalyzed hydrolysis of pNPAc by binding to site I. In contrast, tamoxifen and tetracycline were unable to inhibit esterase activity of HSA.

Conclusions: The enzyme inhibition results suggested that osthole, aspirin and indometacin binding sites on the HSA molecule are the same (Sudlow’s site I). These observations also mean that the large site I pocket on HSA contributes effectively to its promiscuous esterase activity (against aspirin and pNPAc).

Keywords: Human serum albumin, Esterase activity, Sudlow site I, Binding site, Site marker drug
Title:
Structural and Functional Investigation of an Antagonistic Heterodimeric VEGF

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Abstract:
Vascular endothelial growth factor (VEGF) is a dimeric protein that controls much of vascular development through binding, dimerization and activation of its receptor (KDR), resulting in activation of angiogenic cell signaling pathways. Based upon this mechanism, we constructed a heterodimeric variant of VEGF (HD-VEGF) that contained one functional and one non-functional site. HD-VEGF can only bind to monomeric receptors and act as VEGF antagonist. In our previous studies the KDR binding sites of VEGF were precisely determined and replaced by some suitable segments of other proteins. After building a 3-D model of the mutant form and MD simulation, the binding of this variant to KDR was investigated using docking energy landscapes. Based on the constructed model, the modified encoding gene of VEGF receptor binding domain was synthesized. The modified and native VEGF genes were then overexpressed as inclusion bodies in E. coli and refolded together to produce HD-VEGF variant. VEGF heterodimer was purified from VEGF homodimers through two-step affinity chromatography using Ni-NTA agarose and Strep-Tactin columns. Far-UV CD and fluorescence spectroscopy studies showed no significant structural changes in the HD-VEGF in comparison with homodimer variants and confirmed that the formation of heterodimer and its purification were successfully carried out. This variant can significantly inhibit the proliferation and capillary tube formation of endothelial cells in vitro (with IC50 values 33 ng/ml and 24 ng/ml, respectively). Based on these studies, it can be concluded that the HD-VEGF will compete with the native VEGF for receptor binding and antagonizes its action.

Keywords: Antagonistic VEGF, Proliferation assay, Capillary tube formation assay, Far-UV CD, Fluorescence spectroscopy
Title: Helicobacter pylori CagA EPIYA Motifs and Association with Clinical Consequences in Iran

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Abstract:

Introduction: Chronic Helicobacter pylori infection is known to be associated with the development of gastritis, peptic ulcers (PUs), gastric cancer and gastric MALT lymphoma. H. pylori CagA is the first bacterial oncoprotein to be identified in relation to human cancer. The pathogenic CagA protein contains a highly polymorphic Glu-Pro-Ile-Tyr-Ala (EPIYA) repeat region in the C-terminal part of the molecule. CagA diversity with regard to EPIYA-A, -B, -C, or -D phosphorylation motifs may play an important role in H. pylori pathogenesis, and therefore determination of these motifs in H. pylori clinical isolates can become a useful prognostic tool.

Methods: H. pylori strains were obtained from 149 patients with gastritis and 31 patients with PUs referring to the endoscopy units of several cities in Iran. After DNA extraction, these strains were investigated for the presence of cagA gene and CagA EPIYA motifs using PCR amplification. Multiple Linear and logistic regression models were used for the analysis of data using SPSS software.

Results: A total of 121 isolates were CagA positive and varied according to their C-terminal motif. Five different sizes (bp) were observed for this region by PCR (370/AB, 470/ABC, 500, 570/ABCC, 670/ABCCC). The statistical analysis showed no correlation between these motifs and PUs or gastritis (P> 0.05).

Conclusion: It is proposed that the H. pylori CagA EPIYA motifs might not be considered as an important determinant of gastritis or PUs in Iranian population.

Keywords: H. pylori, CagA, EPIYA, Peptic ulcer, Gastritis
Title:
Construction of bivalent and bispecific camel antibody library

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Abstract:
Introduction: After the discovery of camel antibodies that devoid of light chains, these antibodies were considered a useful tool for the diagnosis and treatment of many diseases. The main advantage of the variable domain of camel antibody (VHH), followed by small size and low immunogenicity in humans, and the ability to detect antigens that are inaccessible to others antibodies. In this study, A bivalent and bispecific VHH-VHH antibody libraries Against tumor antigens of SKBR3 was constructed. Several studies have shown that both forms of bivalent antibody affinity and specificity for binding to the antigen much higher capacity than single antibodies are.

Method: The Heavy chain variable region VHH-hing and VHH genes were amplified from cDNA by the Polymerase Chain Reaction (PCR). Hinge of camel IgG2 and IgG3 were used as linkers to connect two VHH together. We connect these fragments by a method based on cloning. pComb3x Vector and VHH-HING insert were digested with Xho1 and Sac1 and then ligated. VHH was transformed into this vector by Xho1 and Spe1. In order to check the diversity of the libraries, RFLP was performed on several samples.

Results: The vector contains bivalent and bispecific antibody transformed into electrocompetent DH5α Escherichia coli yielding a library with 105 total transformants. The difference in cutting pattern fragments in RFLP, reflects the diversity of the constructed library.

Conclusions: The easy generation steps and the biophysical properties of these bispecific and bivalent constructs based on camel single-domain antibody fragments makes them particularly attractive for use in therapeutic or diagnostic programs.

Keywords: camel antibody, bivalent and bispecific antibody, tumor marker.
Title:
Construction of a vector for expression of human glial cell line-derived neurotrophic factor (hGDNF) in mammalian cell line

Authors:
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Abstract:
Introduction: Glial cell line-derived neurotrophic factor (GDNF) which was first purified and characterized in 1993 is a disulfide linked homodimeric glycoprotein. This growth factor promote the survival of the embryonic dopaminergic neurons of the midbrain which is produced in the form of a precursor, pre-proGDNF. Its cDNA encodes a 211 amino acid containing polypeptide that is processed and cleaved into a mature form comprising 134 amino acids. The aim of this study was construction of a bicistronic expression vector containing two copies of hGDNF for stable expression in a mammalian cell line. Method: Coding sequence of hGDNF was determined by bioinformatic studies. Two primer pairs were designed by means of Oligo7.4 software and two coding sequences of the hGDNF were amplified with the signal sequence at their N-terminus and 6xhistidine tag at their C-terminus. Using T/A cloning method, the fragments were ligated into pTZ57R/T vector. After confirmation of accuracy of two amplified sequences, which was cloned in pTZ57R/T, each fragment was digested with suitable enzymes separately, purified and ligated into one of multiple cloning sites of pBudCE4.1/attB, which is a 4.6 kb vector designed for simultaneous expression of two copies of genes in mammalian cell lines. The ligation mixture was transformed intoTOP10 E. coli competent cells. After isolation of positive colonies by colony PCR, the recombinant bicistronic vector was prepared and purified from transformed clones. At last, the accuracy of the vector was verified by digestion method and sequencing and it was co-transfected with a vector expressing phiC31 integrase, which is a sequence-specific recombinase, into HEK293T cell line to study the functionality of the vector for stable expression of hGDNF protein. Results: In this study the expression vector was constructed, and TOP10 E. coli cells were transformed successfully. Then HEK293T cells were transfected and cell clones stably expressing and secreting hGDNF in to the cell medium were isolated. The clones producing the highest amount of GDNF were expanded. Conclusions: Thus we have constructed a vector for efficient and stable expression of hGDNF in mammalian cells. The purity and biological activity of this recombinant protein will be studied in next step.

Keywords: rhGDNF, HEK293T, phiC31 integrase, sequence-specific recombinase
Title:
DNA delivery by a neutral liposomal nanosystem: an introduction to virus-like gene delivery systems

Authors:
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Abstract:
Introduction: Non-viral gene transfer systems for human gene therapy applications represent one of the widest fields of chemical, biological and medical research today. So we attempted in this study to introduce an efficient virus-like system for gene delivery and DNA vaccine production by designing a delivery system based on membrane phosphatidylcholine.
Methods: DNA was encapsulated in bilayer vesicles by employing two contiguous dehydration-rehydration processes. The characteristics of the obtained structure were investigated by various techniques such as electrophoresis, spectroscopy and DLS (dynamic light scattering). These structures were added to CHO cells to deliver GFP reporter gene. Their transfection efficiency was studied using fluorescence microscopy and fluorometry.
Results: the designed lipid bilayer systems which were zwitterionic in terms of surface charges were able to encapsulate DNA with high efficiency (98%) even in the absence of cations, and these results were unprecedented. The resulted system was highly stable, in a way that it was able to keep the DNA molecule in its structure for over 6 months. These virus-like lipid bilayer vesicles were able to transfect the reporter gene GFP to CHO cells.
Conclusions: Due to promising results obtained in this study, it can be expected that cell membrane-based neutral liposomes would be considered in the near future as an alternative to cationic carriers in gene delivery. By mimicking virus functions in fusion to cell membrane and transferring DNA to mammalian cells, these neutral liposomes can be introduced as artificial viruses in gene therapy.

Keywords: bilayer phospholipid vesicle, neutral liposome, artificial virus, gene delivery
Title:
Bioinformatic study of a STR marker in the promoter of Bcl2

Authors:
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Abstract:
Introduction: Bcl2 is one of the most important anti-apoptotic genes that facilitates the survival of tumor cells, Bcl-2 can also protect cells against apoptosis induced by agents with different mechanism of action and can increase resistance to anticancer drugs. Over expression of the anti-apoptotic protein Bcl2 has been associated with several malignancies. By investigating the promoter region of this gene, we can find polymorphisms that lead to over expression of this gene in malignancies.
The aim of this study is to find a Short tandem repeat(STR) in the promoter region of Bcl2 gene by bioinformatic studies.

Methods: Several databases including NCBI, Ensembl, Human genome browser and eukaryotic promoter database(EPD) were used in order to find the promoter sequence and polymorphic markers in the promoter region.

Results: Among the STR's investigated in the promoter region of Bcl2, polymorphic CA repeat was chosen as the STR to be investigated in the labrotary.

Conclusion: Due to the importance of Bcl2 gene in Malignancies, this study has focused on a STR marker in the promoter region of Bcl2, to evaluate its association with Cancer. findings its relationship with cancer can help us in diagnosing people susceptible to cancer and can also have therapeutic purposes.

Keywords: Bcl2, Promoter, Bioinformatic, STR
Title: Inhibitory effects of three synthetic compounds on glycation of human serum Albumin

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Abstract: Introduction: Albumin is the major circulating protein in blood, can undergo increased glycation in hyperglycemia. It has become evident that protein glycation has major impact on protein activity, folding and stability.

Method: In the present study, three synthetic compounds were studied to investigate their antiglycative property in the HSA/Glucose system. The level of glycation and conformational alterations of glycated HSA in presence and absence of extract and synthetic compounds were assessed by congo red assay, SDS-PAGE, fluorescence and circular dichroism spectroscopy.

Results: Significant alteration in the secondary structure of Albumin was observed upon glycation, which was mitigated by applying synthetic compounds. Our results represent the antiglycative property of synthetic compounds and their application for possible treatment of AGE-associated disease.

Conclusions: Our findings provide a strong rationale for further studies and established that mentioned compounds possess antiglycative action.

Keywords: Albumin, Glycation, Synthetic compounds, Antiglycative
Title:
The role of Epstein-Barr virus in Multiple Sclerosis

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Abstract:
Introduction: Both epidemiological and experimental studies provide evidence for an association between Epstein-Barr Virus (EBV) infection and Multiple Sclerosis (MS). This includes the observation that all MS patients show serological markers of past EBV infection. EBV could activate autoreactive T cells by several mechanisms, but it is not clear why this leads to MS. The aim of this study was to evaluate the level of antibodies against EBV in MS patients and apparently healthy individuals.

Method: Sera were collected from 38 patients with primary clinical MS manifestation and 38 healthy individuals as control from the MS Clinic in Alzahra and Kashani Hospitals in Isfahan. The level of Immunoglobulin G (IgG) against EBV capsid antigen (EBV-CA) and Epstein-Barr nuclear antigen 1 (EBNA1) was assessed by using commercially available quantitative ELISA.

Results: Our results indicated that the level of EBV-CA and EBNA1 in all the MS patients’ sera were positive. However, 71% of the controls’ sera showed positivity against EBV-CA and EBNA1 (27 positive subjects and 11 negative subjects).

Conclusions: These finding suggest EBV plays an important role in the pathogenesis of MS but further studies are needed to evaluate its function.

Keywords: Multiple sclerosis; Epstein–Barr virus; viral capsid antigen; Epstein-Barr nuclear antigen 1; ELISA
Title:
Long noncoding RNA ANRIL and its intronic gene, P15 INK4B, revealed different expression patterns in breast cancer

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Abstract:

Introduction and aims: Large noncoding RNAs (lncRNA) have recently introduced as novel tumor-suppressors and oncogenes. Recent studies have revealed mechanistic insight of large ncRNAs regulating key cancer pathways at a transcriptional, post-transcriptional and epigenetic level. INK4 locus on human chromosome 9p21 encodes for three distinct tumor suppressors, p16INK4A, p14ARF and p15INK4B, and is altered in an estimated 30–40% of human tumors. ANRIL is a long noncoding antisense RNA transcript overlapping the INK4B/ARF/INK4A locus. Despite little knowledge regarding ANRIL function, recent evidences have suggested a regulatory role of ANRIL for that locus. Here we examined a potential expression patterns of INK4 locus genes as well as lncRNA ANRIL in breast cancer.

Material and Methods: Breast tumor samples and apparently non-tumor ones have been obtained from Tumor bank of Imam-Khomeini medical center of Tehran University of medical sciences. RNA extracted from the samples using Trizol reagent, cDNA synthesized, and relative expression was measured by quantitative real-time PCR. GAPDH mRNA was also quantified as an internal control, the expression of other genes were normalized to its expression value.

Result and discussion: Quantitative Real-Time PCR results showed lncRNA ANRIL was overexpressed in tumor samples of breast cancer in comparison to non-tumor ones. In contrast to ANRIL, its intronic tumor suppressor gene P15INK4B was downregulated in tumors relative to non tumor specimens. Our finding is an agreement with the previous studies claiming that lncRNA ANRIL inhibited the expression of P15INK4B. To our knowledge, the data presented here is a unique study regarding expression of lncRNA ANRIL and its correlation with P15INK4B in breast cancer. All together our data suggested that missregulation of ANRIL might have a role in tumorogenesis of breast.

Keywords: Key words: long noncoding RNA, ANRIL, INK4 locus, Breast cancer
Title:
The effects of Gibberellic acid and Salt stress on some growth and physiological parameters of tomato (Lycopersicon esculentum Mill.) under In vitro culture

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Abstract:
Introduction: High salinity is one of the most serious threat to plant growth and production. The important purpose of this study is the effect of gibberellic acid on salt tolerance of tomato (lycopersicon esculentum Mill.) under In vitro culture.Method: In this study lycopersicon esculentum Mill. was co-treated with 0, 1 and 2 mgL\(^{-1}\) gibberellic acids (GA\(_3\)) and 0, 100, 150, 200 mM NaCl under in vitro MS culture medium. After two weeks post culture, parameters including stem length, fresh and dry weight, total number of nodes and average length of internodes were measured. Chlorophyll and carotenoid content of leaves were also measured.Results: Results showed that treatment with 1 and 2 mgL\(^{-1}\) GA\(_3\) at concentrations of 0, 150 mM NaCl decreased carotenoid, chlorophyll a, b and total chlorophyll content.In MS medium containing 0, 100, 150 mM NaCl treated with 1 and 2 mgL\(^{-1}\) GA\(_3\), increasing of stem length was observed.In MS medium containing 0, 100 mM NaCl supplemented with 1 mgL\(^{-1}\) GA\(_3\) was applied, the total fresh and dry weight of root was increased.In MS medium containing 100 mM NaCl supplemented with 1 mgL\(^{-1}\) GA\(_3\) was applied, the total fresh and dry weight of the arial part of the plant (shoot and leave) was increased.The most evident increase of the total number of nodes and the average length of internodes has seen in MS medium containing 100 mM NaCl treated with 1 and 2 mgL\(^{-1}\) GA\(_3\).Conclusions: It can be concluded that tomato plants treated with low concentration of GA\(_3\) could increase salt tolerance by changing some physiological responses.

Keywords: Lycopersicon esculentum Mill., Salt stress, Gibberellic acid, In vitro culture.
Title:
A Rapid and Simple Method for Extraction of High Quality Genomic DNA From Paraffin Embedded Tissues

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Abstract:
Formalin fixed paraffin –embedded tissues are an extremely valuable source for genetic studies of cancer and variety of disease. So far many protocols have been introduced for genomic DNA extraction from FFPE, but such protocols remain time consuming and cannot produce high quality DNA. We report a robust and rapid protocol for extracting DNA from FFPE. The UV spectrophotometric and gel electrophoresis analysis resulted in high A 260/A280 ratio (>1.8). Subsequent evaluations were performed using some quality dependent techniques (e.g. PCR and restriction digestions). This protocol requires no enzymatic processing and accordingly its low cost making it an appropriate method for large scale DNA isolation from FFPE.

Keywords: FFPE,DNA,Extract
Title:
The effects of iron on the alleviation of cadmium toxicity in Matricaria chamomilla

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Abstract:
Introduction: Heavy metal stress is an important factor influencing plants growth. Among nonessential heavy metals, Cd is one of the most well-known heavy metals that influence all physiological and biochemical processes in plants. Cd induces disorders in plant growth and development. Some plants can tolerate even concentrations of different metals without visual symptoms of toxicity. For example Matricaria chamomilla is an important medicinal plant which is tolerant to Cd. It has been reported that this plant accumulates high amounts of Cd preferentially in the roots, indicating that it belongs to the group of facultative metallophytes or metal excluders.

Methods: The influence of different concentrations of cadmium and iron were studied on chamomile (Matricaria chamomilla) plants. For this reason, chamomile seeds were planted in greenhouse conditions. Then at the early vegetative stage the plants were transplanted to ½ Hoagland hydroponic solution and after a week of adaptation to the new environment, for 18 days were treated with 120µM and 180µM CdCl$_2$ and also 0.3mM and 0.6mM Fe-EDTA treatments. In this research, root and shoot dry weight, leaf number per plant and root and shoot elongation at the reproductive stage were measured. The results suggested that treatments of appropriate concentrations of iron improved root and shoot dry weight and increased root and shoot elongation.

Result: It is also suggested that the occurrence of Cd toxicity in chamomile plants is mediated by Fe nutrition.

Conclusion: The interaction between Cd and Fe, as obvious in our research, substantiates to be useful for alleviation of Cd toxicity in chamomile seedlings. High Fe nutrition may reduce the Cd transportation through Fe channels, leading to reduced adverse effects of Cd on morphological treats of this plant.

Keywords: cadmium, iron, Matricaria chamomilla, toxicity, growth parameters
Title: Effect of silver nanoparticles and almond bark extract on the physical properties of biodegradable starch-PVA films

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Abstract: Introduction: At present there is an interest in biodegradable edible packaging due to the excellent biodegradability, biocompatibility, edibility and their potential applications. These films may operate as carriers of many functional ingredients, such as antioxidants, antimicrobial agents, flavours, spices and colorants. The aim of this work was to evaluate starch-PVA (polyvinyl alcohol) edible films incorporated with the silver nanoparticles and almond (Prunus amygdalus) bark extract.

Method: Biodegradable films based on starch-PVA and with addition of active agents were prepared using the casting technique. Total phenolic content (TPC), and antioxidant activity (DPPH and FRAP) of the films determined by spectroscopic methods. The tensile strength (TS) [MPa] and percent elongation (E) at break [%] and the thickness of biodegradable films were determined by ASTM standard.

Result: Addition of PVA to film-forming solutions caused an increase in the maximum tensile strength. Mechanical properties of the pristin film was 3.71 Mpa of (TS) - 75.58 % of (E). The addition starch-PVA films containing of silver nanoparticles and extract increased the tensile strength(4.46 MPa of (TS) and 102.43% of (E) ). The silver nanoparticles decrease antioxidant activity (p<0.05), but starch-PVA film containing almond extract showed an increased antioxidant activity ratio to pristin film. The antioxidant activity occurred in a concentration dependent manner. The thickness of biodegradable films was measured and an average value of five measurements was (0.1) mm.

Conclusions: The results showed that starch edible films incorporated with active agent could be used as effective films due to its excellent mechanical properties and antioxidant activities.

Keywords: Edible film, Starch, Silver nanoparticle, extract, antioxidant
Title:
Luminal and antibody bearing gold nanoparticles as a bio-label for detection of hepatitis B surface antigen

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Abstract:
Introduction: Fast detection of hepatitis B virus plays a crucial role in diagnosis and treatment of the disease.
Method: By co-immobilization of anti-hepatitis B surface antigen (anti-HBs-Ag) antibody and luminol on gold nanoparticles (GNPs), a chemiluminescence based immunosensor was developed for the detection of HBs-Ag. In a sandwich type immunoassay method, anti-HBs-Ag was immobilized in polystyrene wells and used as primary antibody and the secondary antibody conjugated to luminol coated GNP as label, then, HBs-Ag was conjugated between primary and secondary antibodies.
Results: Using hydrogen peroxide as oxidant agent and HAuCl4 as catalyst, the chemiluminescent intensity was proportional to the concentration of HBs-Ag in sample. Applying different catalyst showed that HAuCl4 is the most efficient catalyst for this type of detections.
Conclusions: The immunosensor responded toward HBs-Ag in a wide linear range of 0.125 to 30 ng/ml. The proposed method has successfully applied to determine the HBs-Ag in patient sera.

Keywords: Hepatitis B surface antigen, Luminol, Gold nanoparticles, Chemiluminescence, Immunosensor
Title:
Isolation and characterization of serum alkaline phosphatase isoenzymes

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Abstract:

Introduction: Alkaline phosphatases (ALP) are a family of zinc metallo enzymes. It is known that zinc plays a functional role in the catalysis mechanism displayed by the enzyme. Four genes encode ALP isoenzymes in humans, while three ALP genes are expressed in a tissue-specific manner (i.e., placental, embryonic, and intestinal), expression of the fourth one is nonspecific to a single tissue and is especially abundant in bone, liver, and kidney. This isoenzyme is also called tissue-nonspecific alkaline phosphatase (TNALP). Being an important intercellular enzyme, detection of alkaline phosphatase activity is useful tool in molecular biology. Aims of the study: The aim of this research was evaluating the role of various ALP isozymes in unexpected abortion at early stages of pregnancy. In practical section, the activity of the enzyme was measured in serum of a group of women aged 25-35 years at their early stages of pregnancy. Enzyme Assay: ALPase activity was measured by using Stopped Spectrophotometric Rate Determination method. ALP catalyses hydrolysis para-nitrophenol phosphate (pNPP) releasing para-nitrophenol (pNP). The change in absorbance at 410 nm was determined that is indicative of enzyme activity. Total Protein Determination: Protein concentration was measured by Lowery method (and absorbance determined at 595nm using bovine serum albumin (BSA) as standard. Separation of Alkaline Phosphatase Isoenzymes by Gel electrophoresis and Partial Purification of Human ALPase: Using SDS-PAGE, various isoenzymes of ALP were detected in serum of 35 pregnant women at risk of abortion and a similar group as control. The collected samples were subjected to different steps of purification including precipitation by ammonium sulphate (NH₄)₂SO₄, dialysis and DEAE-cellulose ion–exchange chromatography by using gradient elution buffer. Results: It was found that alkaline phosphatase in serum of pregnant subjects had fewer isozymes compared to the subject group. Conclusions: As diagnosis of abortion risk is vital to the necessary care needed at early stages of pregnancy, isozymes of ALP in serum could be used as a diagnostic marker.

Keywords: Alkaline phosphatase, isoenzyme, para-nitrophenol phosphate, pregnant, abortional, ammonium sulphate, dialysis, ion–exchange chromatography.
Title:
Effect of Aging on Stability of Oligonucleotide-Functionalized Gold Nanorods

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Abstract:
Introduction: Remarkable optical properties of gold nanorods (GNR) have been vastly used in biosensing and biomedical applications. In the light of sensing applicability, GNR based nanobiosensors are functionalized by various biomolecules such as peptides and oligonucleotides, that are known as nanoprobe. However, great sensitivity of GNRs to trace environmental changes and tendency to non-specific aggregation makes a crucial step in design and production of nanoprobe. Therefore, optimization of biorecognition element and stability investigations of nanoprobe is of great significance. Herein, stability of aged samples of oligonucleotide-functionalized GNRs has been studied at ambient temperature.

Method: Gold nanorods were synthesized according to previously reported seed-mediated growth method. The nanostructure was purified by centrifugation and redispersed with a fixed concentration (1 OD). Surface of GNRs was biofunctionalized by a thiolated 22-mer oligonucleotide (1nM) after concentration optimization. Surface plasmon resonance (SPR) and Fourier transform infrared spectroscopy were used to monitor the bioconjugation via formation of Au-S bond. Samples of nanoprobe were then aged at ambient temperature (25 ºC) for different time intervals. Stability of the aged nanoprobe was investigated by monitoring the SPR bands of the nanostructure.

Results: The characteristic SPR bands of GNRs along the short and long axis appeared at 520 nm and 724 nm, respectively. There was negligible shift in the wavelength maxima of nanoprobe after one week aging, with little decrease of intensity for both transverse and longitudinal surface plasmon resonance.

Conclusions: The oligonucleotide-functionalized nanoprobe is stable at ambient temperature for biorecognition purposes.

Keywords: Gold Nanorod, Aging Process, Nanoprobe, SPR
Title:
Rapid and non-invasive diagnosis of the presence of coronary artery disease based on 1H-NMR spectra of human blood plasma using supervised self-organizing map

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Abstract:
Coronary artery disease (CAD) is a major cause of mortality and morbidity in developed countries, affecting as many as one in three individuals before the age of 70 years [1]. Despite a slight decrease in prevalence that has occurred over the past decade, it still contributes to nearly 15% of deaths [2]. Although a wide range of risk factors for coronary artery disease have been identified from population studies, these measures, singly or in combination, are insufficiently powerful to provide a reliable, noninvasive diagnosis of the presence of coronary artery disease [3]. Techniques such as genomics, proteomics and metabonomics (a systems approach to examining the changes in hundreds or thousands of low-molecular-weight metabolites in an intact tissue or bio fluid) offer the prospect of efficiently discriminating individuals who have special disease or toxic states. The NMR based technique offers several clear advantages in the clinical goals. It can be carried out on standard preparations of serum, plasma or urine samples [4-6]. In this work, supervised Kohonen artificial neural networks (SKANN) as a supervised self-organizing map (SOM) [7-12] is used as a non-linear modeling and visualizing method to analyze proton nuclear magnetic resonance (1H-NMR) spectroscopy data which obtained from human blood plasma samples in a case control research on coronary artery diseases (CAD). The goal of this study is human disease diagnosis and classifying CAD samples on basis of visualizing the relationship between different spectral patterns. Samples were collected after angiography and included patients with CAD and non-findings individuals (healthy cases) (64 samples with 2 classes). In this study, the partial least squares (PLS) [13, 14] as a linear two-way analysis chemometrics approach was used to reduce dimensionality of data. To enhance the classification performance, genetic algorithm (GA) applied on PLS latent variables to select discriminant PLS factors. In the next step, the oblique rotation (OR) [15] was implemented on the selected factors to transform them linearly to obtain the optimal discriminative factors. The optimal factors were introduced as input to SKANN [9-12] and also, partial least squares-discriminant analysis (PLS-DA) [11, 16] as non-linear and linear classification methods, respectively. Classification results were calculated and evaluated by cross validation and external test set and compared together. The best result obtained using SKANN. All the training and cross validated samples were successfully classified using proposed method. Percent of correct classified samples for test samples were 0.92 and 0.86 using SKANN and PLS-DA, respectively. It can be concluded that the combination of chemometrics approaches and 1H-NMR spectra of human blood plasma can be used as a powerful diagnostic tool to detect CAD and similar diseases which cause changes in metabolic contents of human blood.

Keywords: Classification (Chemometrics), Supervised self-organizing map, Nuclear magnetic resonance spectroscopy, Genetic Algorithm, PLS-DA.
Title: Lysozyme interaction with two types of biocompatible polymers as ophthalmic drug delivery systems

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Abstract: The unique physical properties of hydrogels have created particular interest for their use in drug delivery applications. Their highly porous structure permits loading of drugs into the gel matrix and subsequent drug release at a rate dependent on the diffusion coefficient of the small molecule or macromolecule through the gel network. Low molecular weight proteins (LMWPs), such as lysozyme, may be suitable drugs to be entrapped into the hydrogel contact lenses. Lysozyme is a small enzyme that attacks the protective cell walls of bacteria protecting us from the danger of bacterial infection. In practice, polyacrylamide and pHEMA Hydrogel discs were immersed into the lysozyme/phosphate buffer solution and the enzyme was loaded into the gel at the temperature of 37°C and pH 6.2. The hydrogel prepared without and with lysozyme were studied by various techniques including, differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). SEM image of a hydrogel loaded with lysozyme showed that its structure is differed from pure hydrogel, which pointed transparency decreases insignificantly. DSC study showed an increase in Tg of hydrogel after blending with lysozyme. It was found that tensile strength increases with presence of lysozyme in the hydrogel membranes.

Keywords: Ocular Drug delivery system, soft contact lens, polyacrylamide, 2-Hydroxyethyl methacrylate, lysozyme
Title:
A novel superoxide dismutase mimic based on copper (II) complex

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Abstract:
Introduction: Superoxide dismutase (SOD) is an antioxidant enzyme involved in scavenging of superoxide and protects cells from oxidative damage. Unfortunately, natural enzyme can be easily denatured by environmental changes (such as temperature, pH and so on). Thus, tremendous efforts have been devoted to develop artificial SOD mimetics. In this study, copper (II)-cysteine complex (Cu-Cys) was synthesized and its SOD mimetic activity was investigated by pyrogallol autoxidation assay and electrochemical techniques.

Method: Pyrogallol autoxidation method was used for assaying SOD mimetic activity of Cu-Cys complex. Then, an application test for this novel SOD mimetic was performed by fabricating a novel biomimetic superoxide sensor based on electrochemical method.

Results: According to pyrogallol autoxidation method, Cu-Cys complex exhibited good SOD mimetic activity and followed a typical Michaelis-Menten behavior. The amperometric response of superoxide was monitored at a carbon paste electrode modified with Cu-Cys complex in potential 250 mV (vs. Ag/AgCl) and pH 7.4 phosphate buffer 0.1 M. The linear detection range and detection limit of superoxide at Cu-Cys complex modified carbon paste electrode were 11.3-316.4 and 5.3 µM respectively. The sensitivity of this superoxide sensor was 21.1 (nA/µM). Hydrogen peroxide, uric acid and citric acid showed no interference effect on the electrode performance.

Conclusions: This remarkable performance indicates that Cu-Cys complex may be an efficient SOD mimetic candidate for constructing biomimetic superoxide sensor for clinical and/or industrial applications.

Keywords: Mimetic, Superoxide dismutase, Biomimetic superoxide sensor, Cysteine
Title:
Modeling human 5-alpha reductase by molecular dynamics method in order to design inhibitor

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Abstract:
Introduction: Dysfunction in the function of 5-alpha reductase enzyme, that converts testosterone to dihydrotestosterone, is responsible for disruptions in the body in the different age groups, such as male pattern hair loss (MPHL) in men. Inhibitors of both isoenzymes of this enzyme can be used as medicine, but due to the lack of three-dimensional structure of this transmembrane enzyme, designing inhibitors for this enzyme is difficult. The purpose of ongoing project is designing an inhibitor for this enzyme. Method: MODELLER version 9.2 was used to build homology models of 5-alpha reductase isoenzymes (I and II). From the 1000 model generated with MODELLER for each isoenzyme, the one corresponding to the lowest value of the probability density function (pdf) was selected for further analysis. Models evaluated with Procheck and Verify3D and in selected model for isoenzyme I, only %1.4 of the residues were in disallowable Ramachandran plot and in selected model for isoenzyme II, only %0.4 percent of the residues were in disallowable Ramachandran plot. The models obtained in these steps were used as starting structures for molecular dynamics simulation. Thirty nano second molecular dynamics simulation at 300 K was performed for each isoenzyme. Results: The values of root mean square deviation (RMSD), radius of gyration, potential energy and kinetic energy at the last 15 ns of simulation were reported in the following table.

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>Root mean square deviation(RMSD) (Å)</th>
<th>Temperature (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10.21(±0.2)</td>
<td>299.97±0.94</td>
</tr>
<tr>
<td>II</td>
<td>5.54(±0.14)</td>
<td>300.02±1.62</td>
</tr>
<tr>
<td>Radius of gyration (Å)</td>
<td>20.78(±0.19)</td>
<td>17.91(±0.08)</td>
</tr>
<tr>
<td>potential energy(kJ/mol)</td>
<td>-1574289(±1332)</td>
<td>-576053(±822)</td>
</tr>
<tr>
<td>kinetic energy(kJ/mol)</td>
<td>287655(±903)</td>
<td>108597(±585)</td>
</tr>
<tr>
<td>Total energy (kJ/mol)</td>
<td>-1286634.4(±1656)</td>
<td>-467456(±1083)</td>
</tr>
</tbody>
</table>

Conclusion: Small variations in potential and kinetic energy during the last 15 ns of MD simulation and small changes in temperature show thermal equilibrium in systems, and the simulations time were sufficient. The final structures obtained from molecular dynamics simulation were used for docking of inhibitors and design a new inhibitor for this enzyme.

Keywords: 5-alpha reductase enzyme, Modeling, modeller, molecular dynamics simulation.
Title: investigation of manganese influence on Matricaria chamomilla under cadmium stress

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Abstract: Introduction: Heavy metal stress is an important factor influencing plants growth. Among nonessential heavy metals, Cd is one of the most well-known heavy metals that influence all physiological and biochemical processes in plants. Some plants can tolerate even concentrations of different metals without visual symptoms of toxicity. For example *Matricaria chamomilla* is an important medicinal plant which is tolerant to Cd.

Method: In this research the effect of different concentrations of cadmium and manganese on *M. chamomilla* were studied. The aim of our study was to investigate the effects of Mn on Cd toxicity alleviation. For this reason, chamomile seeds were planted in greenhouse conditions. Then at the early vegetative stage the plants were transplanted to ½ Hoagland hydroponic solution and after a week of adaptation to the new environment, for 18 days were treated with various concentrations of Cd and Mn. In this research, root and shoot dry weight, number of leaves per plant, root and shoot elongation, chlorophyll a and b contents at the reproductive stage were measured.

Results: The results indicated that treatments of 0.3 mM and 0.6 mM Mn along with 180µM and 120µM CdCl₂ improved root and shoot dry weight and increased root elongation rather than 180 µM and 120µM CdCl₂ treatments. The highest total chlorophyll content was observed in 0.3 mM Mn treatment.

Conclusion: Based on our findings, the interaction between Cd and Mn substantiates to be useful for alleviation of Cd toxicity in chamomile seedlings. The chlorophyll amount declined with increasing Mn concentrations and attained its maximum in 0.3mM Mn concentration for all mentioned treatment.

Keywords: cadmium, manganese, Matricaria chamomilla, growth parameters, chlorophyll
Title:
Theoretical study on the intramolecular hydrogen bond in nitro-substituted naphthazarin

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Abstract:
Introduction: Currently, there is considerable interest in the study on naphthazarin (hereafter NZ) and its derivatives because of biological importance (e.g. antitumor and antiviral activity, wound healing, antimicrobial, antithrombotic properties and their use in the development of cardioprotective preparations). This compound has been extensively studied theoretically and experimentally. The aim of the present work is to demonstrate the effect of nitro substitutions of NZ on the intramolecular hydrogen bond strength.

Method: All calculations were performed using Gaussian03 and NBO 5.0 programs. The full geometry optimization of NZ and its NO2 substitutions were performed with DFT calculations at the B3LYP/6-31G** theoretical level. Wiberg bond orders were calculated using NBO 3.0. The second order interaction energies, orbital population and natural steric analysis were performed at the B3LYP/6-31G** level using NBO 5.0 program, ¹H chemical shift was calculated at this theoretical level by GIAO method.

Results and discussion: Performed calculations on the 9 derivatives of NZ show that the substitution of nitro increases the O…O and O…H bond length, and decreases OH…O bond angle and ¹H chemical shift. By natural bond orbital (NBO) method, the effect of substitution on the hydrogen bond strength, the charge distributions, steric effects, and electron delocalization in the studied compounds were investigated. NBO analysis are in good agreement with the calculated results the geometrical parameters.

Conclusions: All performed calculations indicate that the electron-withdrawing effect of NO2 decreases the hydrogen bond strength. The geometrical parameters are in good agreement with the NBO analysis and the proton chemical shift results.

Keywords: intramolecular hydrogen bond, DFT, nitro naphthazarin
Title:
A new approach for In vitro refolding of Arabidopsis SAL1 inositol polyphosphate 1-phosphatase

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Abstract:
SAL1 in Arabidopsis thaliana is an abiotic stresses responsive gene encoding a bifunctional enzyme that possesses both inositol polyphosphate 1-phosphatase and 3', (2')5'-bisphosphate nucleotidase activities. The inositol polyphosphate 1-phosphatase activity of SAL1 is involved in catabolizing inositol 1, 4, 5-trisphosphate (IP3) which participates in the pathway of phosphoinositide signaling. Moreover, SAL1 is known as a repressor of posttranscriptional gene silencing (PTGS) through removal of 3'-phosphoadenosine 5'-phosphate (PAP). Hence, SAL1 is an important protein playing an important function in cell signal transduction. In order to perform further investigations on SAL1, the gene was first overexpressed as inclusion body in E.coli and then purified and refolded. Briefly, to refold SAL1, cell pellet was re-suspended in TE buffer layered on top of 50% glycerol and centrifuged. The supernatant, aspirate away from the refractile body pellet. The pellet re-suspended in TE + 5 mM EDTA (pH=8.0), and then added to denaturation buffer (8 M Urea/ 20 mM Tris/ 10 mM DTT), and mixed gently at RT for 1 hours. The sample was centrifuged, diluted with 9 volume of 20 mM Tris (pH=8.0) + 10% Glycerol (Rapid dilution), and then incubated at 4° C for 1 hours. The samples were centrifuged and subsequently the supernatant was dialyzed against dialysis buffer (20 mM Tris (pH=8.0)/ 0.5 mM DDT/ 10% Glycerol). The refolded and purified SAL1 protein was centrifuged at 12000 rpm and its inositol polyphosphate 1-phosphatase activity was assayed using IP3 substrate. The results showed that the protein was folded successfully and can properly hydrolyze IP3.

Keywords: SAL1, Refolding, Inclusion body, Inositol polyphosphate 1-phosphatase, IP3
Title:
Theoretical investigation for Rotavirus detection by carbon nanotube

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Abstract:
Rotaviruses cause approximately 111 million episodes of gastroenteritis per year including 25 million clinic visits, 2 million hospitalizations and 600,000 Rotavirus-related deaths in children under the age of five years worldwide and Clinical methods are limited yet for the treatment and detection of rotaviruses. Rotavirus structural proteins have key roles in rotavirus interactions with other molecules. In this study we examined Vp7 protein of rotavirus interaction with singlewalled carbon nanotube (SWCNT) to developed new method for Rotavirus detection. In this investigation, we carried out a theoretical study on Vp7 interaction with SWCNT by quantum mechanical (QM) method using density functional theory (DFT) with B3LYP keyword; wherein the charge changes and atomic forces have been described using this method. So, by investigation of the physical properties of interaction such as energy of interaction, charge distribution in place of interaction the predominant binding force in place of interaction has been proposed. Our results confirmed that SWCNT interaction with VP7 has adequate stability in solvent acceptable energy level. The results also show that non-covalent binding forces in place of interaction are predominant so that changing in chargedistribution caused by VP7 interaction with SWCNT. The present study confirmed that VP7 interaction with SWCNT can be a new way for rotavirus detection.

Keywords: Rotavirus, Vp7, SWCNT, binding force
Title: Docking and Molecular Dynamics Simulation Studies of Interactions between Cyclooxygenases Enzymes and Celecoxib drug

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Abstract: Introduction: The major enzymes responsible for the synthesis of Prostaglandins (PGs) are cyclooxygenases (COXs) which have been identified into two isoforms, known as COX-1 and COX-2. COX-1, the pre-dominantly form of the enzyme, is expressed throughout the body and performs a number of homeostatic functions, while COX-2 expression is associated with inflammation, pain and other pathologies such as cancer proliferation. Both enzymes are sensitive to inhibition by conventional Nonsteroidal Anti-Inflammatory Drugs (NSAIDs). Efforts have been done to develop COX-2 selective inhibitors in order to reduce the classical side-effects. In this study, we have employed homology modeling, molecular dynamics (MD) simulation, and molecular docking to analyze the interactions of Celecoxib (selective COX-2 inhibitor) with both COX enzymes and specify their effective binding sites. Method: The amino acid sequences of COXs, taken from the NCBI Web site were loaded to the SWISS-MODEL server (A fully automated protein structure homology-modeling server) to obtain 3D structures of the receptors. The crystal structures of 1Q4G and 3NT1 were obtained as templates for COX-1 and COX-2, with sequence identities of 93.85% and 88.2% respectively. Resulting 3D structures of COX-1 and COX-2 were submitted to Gromacs simulation package for energy minimization and molecular dynamics simulation. Two separate simulations, each for 10 ns were carried out on COX-1 and COX-2 at 310 K. Both enzymes were first dissolved in water and the systems were neutralized by adding suitable ions. Analysis of RMSD plots and Gyration radiuses confirmed that final structures are equilibrated. On the other hand, the Celecoxib structure was drawn by GaussView and optimized by Gaussian 03. We used HF method and 3-21G basis set for optimizing this drug. Finally, the molecular docking of Celecoxib to COX enzymes were carried out by AutoDock Vina. 9 docking iterations were performed for this ligand with each COX enzyme. AutoDock was employed to determine the free energy of binding. Molegro Virtual Docker package was used in order to analyze the interactions of studied enzymes with Celecoxib. Results: The main purpose in drug-receptor docking is to specify the effective interactions of drug with various aminoacids of the receptor. In this study the main enzyme residues (responsible for interactions between COX enzymes and Celecoxib drug) and their respective total interaction energies, were obtained and tabulated using the Molegro Virtual Docker. These results can be used in designing new derivatives of Celecoxib as potent and selective COX-2 inhibitors.

Keywords: Cyclooxygenase, Molecular Dynamics Simulation, Docking, Celecoxib, NSAIDs.
Title:
A comparisons between Ward’s methods of clustering and similarity matrix to mine any probable influence of tear proteome purification methods on 1DE separation patterns

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Abstract:
Introduction: Digital image analysis quantifies gel lanes in a mathematical manner then different statistical methods can be applied to compare different lanes. Similarity matrix and Ward’s clustering methods are popular techniques in this way. Considering tear as a valuable sample in ophthalmology and need for basic information on tear proteome profiling, we conducted the mentioned methods on 1D-proteome profiles to gain inter-specific polymorphism degrees caused by different treatments.
Method: Tear samples obtained with Schirmer strips participating twenty volunteers. They pooled and homogenized to avoid intra-individual variability biases. Eight precipitation methods (acetone; acetone/methanol; TCA/acetone; ammonium sulfate (50, 70,90%) TBP and chloroform/methanol) applied on separate batches and each sample separated by 1D-SDS PAGE according to Laemmli’s system. Silver stained banding patterns were digitalized by a GS-800 densitometer. Quantity One software applied to seek quantitative comparisons. Detected bands were matched in different lanes by tolerance set at 6.5. Based on previous refinements similarity comparison investigated through a similarity matrix (using the Dice coefficient) and clustering methods (using WPGAMA and UPGAMA).
Results: Results of Ward’s (UPGAMA and WPGAMA) clustering methods presented in two separate phylogenetic trees. The tree patterns were the same for WPGAMA and UPGAMA clusters but two branches were longer in UPGAMA clustering. Similarity percentage between two purification procedure presented in an eight*eight matrix were also consistent with the clustering methods.
Conclusions: This study shows that all statistical methods are plausible and somehow similar to mine our proteome samples. They also reflect effect of distinct chemistry of purification reagents on 1D-proteome analyses.

Keywords: Tear proteome, Sample treatment, Clustering, UPGAMA, WPGAMA
Title:
Molecular dynamics study of structure-function relationships in two mutations of mnemiopsin from Mnemiopsis leidy

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Abstract:
The photoprotein Mnemiopsin from Mnemiopsis leidy is a bioluminescent complex formed with the apoprotein and prosthetic factor, emits light in the presence of Ca$^{2+}$ decomposing into apomnemiopsin, coelenteramide and CO$_2$. In order to understand mechanism of the reaction, the study of structure-function relationships was undertaken with respect to two single mutants, Leu36His and Phe186His located in the substrate binding cavity. Our experimental results showed that these mutations stop bioluminescent reaction. Investigation of this mutation with Molecular dynamic simulation indicated an increase in overall flexibility of Ca$^{2+}$-binding loops and substrate binding cavity residues in mutants compared to the wild type. Further analyses on Phe186His mutant revealed that mobility of coelentrazine in some regions and substrate-protein interaction energy is decreased, while these structural properties are not changed in Leu36His mutant. Finally these findings revealed that these two substitutions prevent disruption of peroxide group of coelentrazine and therefor stop bioluminescent reaction by altering the dynamic and energetic properties in this region.

Keywords: mnemiopsin, bioluminescent, molecular dynamic, structural-function relationship
Title:  
Reconsidering the solvent based approach to remove albumin from serum  

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Abstract:  
Introduction: Proteomic analysis of sera and the quest for identifying serum proteins as disease markers have often been hampered by the predominance of several highly abundant proteins including albumin and immunoglobulins. We have been able to refine the albumin depletion protocols and establish a albumin removal method using trichloroacetic acid / organic acid. Method: the efficacy of polarity based solvent effect was studied and changes in major protein bands were monitored by 1-D SDS-PAGE followed by conventional densitometry. Result: In this report, a view on the theoretical basis of the TCA/organic solvent methods has been offered through introducing new parameters which enable us to predict the result of precipitation and choose the best way to handle different kind of samples to improve their 2D map resolution. A new parameter as "signed magnitude of the hydrophobicity change" in response to the pH lowering for an amino acid residue is defined and exploited to categorize standard amino acids in three distinctive groups based on the mode of hydrophobicity change during pH dropping from 7 to 2. Another new parameter as "discriminative factor", employed to examine the abundant constitutive proteins of the serum for their hydrophobicity change tendency upon pH lowering which is considered as an intrinsic protein characteristic parameter. Among serum abundant proteins only three fatty acid binding proteins have the positive d values and serum albumin is successfully highlighted as an exceptional serum component which tends to be removed in organic solvent. Conclusion: The modified TCA/dioxan approach can offer a rapid method for purifying albumin from serum and make it possible to remove albumin masking of the low abundant proteins.  

Keywords: Albumin removal / Trichloroacetic acid precipitation / Discriminative factor / signed magnitude of hydrophobicity change.
Title:
Optimized expression of a bispecific antibody(TaFv) against CD4 and anti-leptin receptor

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Abstract:
Introduction: Mice with leptin(Ob) deficiency or dysfunction in leptin receptor (ObR) signaling show resistance against autoimmune diseases. High serum leptin levels are associated with high incidence of autoimmune diseases and lowering leptin levels ameliorate the diseases symptoms. On the other hand leptin is a multifunctional hormone/cytokine with profound effects on metabolism, reproduction and energy balance. Therefore, blocking all the leptin receptors will probably result in unfavorable effects. Targeted ObR blocking on specific immune cells such as T helper cells may be advantageous for patients with autoimmune diseases. Bispecific diabody (TaFv), with ability to bind and block human ObR and bind to CD4 simultaneously has been produced by this group previously. The aim of this study was to optimize the production of this TaFv using different vectors, hosts, media composition etc. Method: Different experimental conditions were designed using statistical software, were assessed. The expression vector of the gene encoding TaFv was transfected into E.coli HB2151. After growth, single colonies were chosen to be inoculated into LB, 2xTY, and TB medium and were incubated overnight at 37C with shaking. The expression was induced with 1 mM IPTG overnight at 23C after eliminating glucose from media. The supernatant, cytoplasmic extracts and whole cell lysate was extracted using centrifugation, sonication and freeze and thaw cycles. Protein expression was assessed in all fractions using western blot, dot blot and ELISA. Results: High levels of TaFv expression were detected in LB, 2xTY, and TB media in all fractions including, supernatant, periplasmic and cytoplasmic extracts. Conclusions: The optimized culture condition can increase the expression of TaFv, which may facilitate subsequent production, purification and functional study of TaFv protein which might have potential therapeutic applications in autoimmune disorders.

Keywords: TaFv, leptin, expression, autoimmune diseases, E.coli
Title:
Gold nanorods for enhanced delivery of lapatinib in Breast cancer

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Abstract:
Introduction: Breast cancer (BC) is the most frequent cancer in women that results in overexpression of human epidermal growth factor receptor2 (HER2) for 15–25% of patients. Trastuzumab, as a recombinant humanized monoclonal antibody, is used to inhibit the growth signaling pathway via binding to the extracellular domain of HER2. In the case of trastuzumab-resistant BC patients, lapatinib could be employed as an oral small-molecule EGFR/HER2 dual tyrosine kinase inhibitor. However, lapatinib has some limitations to treatment of BC patients. In the present work, gold nanorods (GNRs)-based drug delivery systems have been developed to overcome limitations of lapatinib and improve drug safety and efficiency.

Method: After synthesis of GNRs and their characterization using scanning electron microscopy (SEM) and zeta potential, the GNRs-drug delivery system was prepared by conjugating GNRs with lapatinib. Then the prepared nanosystem was used to treatment of SKBR3 and MCF7 cell line to evaluate efficiency of conjugated lapatinib.

Results: The results demonstrated a GNRs size-dependent efficiency of conjugated lapatinib. Also, in comparison to free lapatinib higher drug delivery was observed because of enhancement in overall lapatinib solubility.

Conclusions: conjugation of lapatinib with GNRs can improve anticancer efficiency of this drug. The conjugated system shows susceptibility to treatment of trastuzumab-resistant BC patients.

Keywords: Gold nanorod, Lapatinib, Breast cancer
Title:
MDS study on the effect on the E276Q mutation on Hepatocyte nuclear factor4 ligand binding domain structure and stability

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Abstract:
Introduction: Hepatocyte nuclear factor4 (HNF4), a member of the nuclear receptor superfamily, is a crucial regulator of a large number of genes involved in glucose, cholesterol, and fatty acid metabolism. Mutations in this protein are responsible for maturity onset diabetes of the young1 (MODY1). An important example of these mutations is the E276Q one, occurring in the HNF-4a E domain. In this study, we have investigated the effects of this mutation on the structure and stability of HNF-4a, by means of in silico tools.
Method: We constructed a structural model of the missense E276Q mutation based on its homology to the X-ray crystal structure of the HNF4a ligand binding domain (PDB code: 1PZL) with MODELLER9v8. The model consists of residues 138 to 368 and includes helices 1 to 12. The final model obtained by molecular mechanics and dynamics methods was assessed using PROCHECK. MD simulations lasting 10 ns were performed for the wild type and mutant proteins in 300K and 500K. We compute RMSD, RMSF per residues, radius of gyration, energies and solvent accessible surface area.
Results: Our structural model revealed a potential energy of mutant decreased in both temperatures. Moreover, the simulation result demonstrates that hydrophilic solvent accessible surface area of mutant increased in 300K.
Conclusions: Experimental results are controversial as to the effect of this mutation on the stability and function of the E276Q mutated protein. Our results are in accordance with a report that has demonstrated higher stability of the mutant protein.

Keywords: HNF4, MODY1, E276 mutant, MD simulation.
Title:
Isothermal Titration Calorimetry Study of Heme-Imidazole Complex in Camel β-casein as a Caseoperoxidase

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Abstract:
The heme group has fundamental role in activation of hemo-enzymes such as horseradish peroxidase (HRP). An alternative approach to the preparation of robust biocatalysts consists in the encapsulation of metalloporphyrins into water-soluble hydrophobic pocket that mimic the polypeptide envelope, which protects the catalytic center of natural enzymes. Artificial enzymes could biomimetically constructed from native protein as host for prosthetic active site to simulate the catalytic functions exhibited by natural enzymes.

In this report, to gain more quantitative insights to study of the structure of caseoperoxidase, the calorimetric (ITC) binding of heme-imidazole to camel β-casein-SDS (3.48 µM/0.96 mM). According to two binding set program fitting of the binding curve (heat of each injection as a function of the heme-imidazole/Cβ-casein-SDS molar ratio), indicates the enthalpy (∆H) of the first binding set is -9.30 ×105 cal/mol, and the ∆H of the second bindingset is -4.54 ×105 cal/mol. Both binding sets were associated with an exothermic net ∆H, indicating that the long-range electrostatic interactions between the charged regions of the heme-imidazole complexes (especially iron (III)) and the ionic regions of the protein/SDS were strong. The heats of each binding set comprised the sum of both electrostatic and hydrophobic interactions. Therefore, the first binding set with higher binding constant (Kb), associated with a more exothermic enthalpy, corresponded to the electrostatic interactions of the heme-imidazole complex more closely to the charged regions of the protein/SDS. Whereas the lower exothermic ∆H, match to the second binding set, was associated with the more hydrophobic interactions involved in accommodating the heme-imidazole complexes with the hydrophobic regions of protein/SDS as an apoprotein. The maximum number of heme_imidazole complexes that could be incorporated into each apo-enzyme (N) in the first and second binding set is about 4.6. Thus, the constructed biomacromolecule with multiple active sites is a multi-enzyme system.

Keywords: Caseoperoxidase, Biomimetic, Artificial enzyme, Horseradish peroxidase (HRP), Camel β-casein, Sodium Dodecyl Sulfate (SDS), Imidazole, Heme, ITC
Title: Status of researches in Biological sciences

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Abstract: The share of research and scientific productions in the field of life sciences in developed countries are about 55 percent, which highly reflects the importance of biological sciences in the present world. According to the number of scientific documents indexed in the Science Citation Index-Expanded (SCIE) of the institute for scientific information of Thomson Reuters during last 20 years (1993-2012), the number of Iranian scientific documents was 138963 (0.61% of the world value). By increasing the number of scientific documents, special in the science section, Iran had a rank of 19 for total scientific production in the world in 2012. Chemistry, Engineering and Physics by contribution of 17.7, 16.6 and 8.8%, respectively, were the most productive subjects. Biochemistry & Molecular Biology by contribution of 2.8% (3907 documents), Plant Science by contribution of 1.9% (2587 documents), Biotechnology & Applied Microbiology by contribution of 1.8% (2491 documents), Cell Biology by contribution of 0.8% (1138 documents), Biophysics by contribution of 0.6% (837 documents) and Zoology by contribution of 0.5% (659 documents) were our most productive in biological sciences. USA, Canada, England, Germany, Australia and France are the first six countries had collaboration with our country to publish scientific documents in bioscience. Documents analyzed form life science researches showed the higher impact factors relative to any other science researches in Iran as the same as in the world. The results indicated an average four for citations per paper in the field of bioscience in Iran that clearly reflects the growth of quality in biological sciences.

Keywords: Science production, Biological science ranking, Iran scientific documents, Scientific cooperation, Scientific contribution, Institute for scientific information
Title:
Detection of SEN virus in Hepatitis B and C infected individuals in Yazd province, Iran by nested PCR.

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Abstract:
Introduction: A recently discovered DNA virus (SEN virus) has been assumed to be responsible for post transfusion hepatitis in humans. SEN virus is blood-borne, single-stranded, non-enveloped DNA virus. Phylogenetic analysis of the SEN virus has revealed the existence of 8 different genotypes (A-H). Two of its strains (D and H), appear to be associated with non-A-to-E hepatitis more frequently than the others. The prevalence of SEN virus in healthy individuals, including blood donors, differs markedly by geographic region. The aim of the present study was to investigate the frequency of SENV-D and SENV-H genotypes in hepatitis B and C infected individuals in Yazd province.

Method: Serum samples derived from 6 HIV/HCV co-infected, 50 HBV infected and 50 HCV infected individuals were examined for SENV-D and SENV-H viraemia by nested PCR. Fisher's Exact Test was used for statistical analyses.

Results: SENV-D was detected in 9 out of 50 (18%) HBV infected, in 5 out of 50 (10%) HCV infected. It was not detected in any of the HIV/HCV co-infected individuals. SENV-H was detected in 2 out of 6 (33.3%) HIV/HCV Coinfected individuals, in 32 out of 50 (64%) HBV, and in 19 out of 50 (38%) HCV infected individuals.

Conclusions: Compare to SEN-D, the frequency of SEN-H was significantly (P< 0.001) higher in both HBV and HCV infected individuals. Although the number of HIV/HCV infected individuals tested were low, but the detection of only SEN-H in them confirms the higher frequency of the SEN-H genotype in the population studied.

Keywords: SEN-V, SENV-D, SENV-H, HBV, HCV, PCR
Title: The Effects Of TiO2 Nanoparticles On Inhibition And Stimulation Of MCF7 Cells And Human Endometrial Adult Stem Cells

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Affiliation: Institute of Biochemistry and Biophysics, Tehran University, Tehran, Iran

Abstract: The purpose of this study is to examine the in vitro cytotoxicity of TiO2 nanoparticles (TiO2NPs) on breast cancer cells MCF7 cells and the human endometrial adult stem cells via MTT cell viability assay and Flow cytometry. Growth inhibitory and apoptosis effects of TiO2NPs toward MCF7 after 48 h were measured. The results demonstrate that TiO2NPs have growth inhibitory and inducing apoptosis against MCF7. In contrast, TiO2NPs could stimulate growth of the human endometrial adult stem cells up to 40% with the same concentration used for MCF7 cell line after 48 h. Consequently, our results show that TiO2NPs enable to act as growth inhibitor for MCF7 cells and growth stimulator for the human endometrial stem cells. This study may offer useful information to design better anticancer compounds for cancer therapy.

Keywords: MCF7 cells, TiO2NPs, The human endometrial stem cells, MTT assay, Flow cytometry
Title:
Peroxidase Isoenzymes from Tea leaves (Camellia sinensis L.)

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Abstract:
Peroxidases (donor: H2O2 oxidoreductase, EC 1.11.1.7; PODs) are glycoproteins with ubiquitous distribution in the plant kingdom, showing generally several isoenzyme forms, high thermo-resistance and activity regeneration after heat inactivation. The multiple isoperoxidase forms found within the same plant source can differ with respect to molecular mass, isoelectric point, pH and temperature optima. They catalyze the oxidation of various electron donor substrates such as phenols and aromatic amines in the presence of hydrogen peroxide. In this study we determined isoenzyme of POD from tea leaves. To determined isoenzymes, leaves of tea harvested in the territory of the Lahijan (in 300 hight), Iran were used. The leaves of tea were homogenized 50mM phosphate buffer (pH7.8) containing 10% polyvinyl polypyrrolidone (PVPP), 1% (v/v) Triton X-100, 2 Mm EDTA and DTT. Peroxidase activity was assayed spectrophotometrically in 470nm using guaiacol as substrate. Total protein content was measured according to the Bradford method using bovine serum albumin (BSA) as standard.

Activity and The number of isoenzyme of POD determined using 12% nondenaturing polyacrylamide gel. Peroxidase bands were detected by immersing the gel in a solution of phosphate buffer containing guaiacol and H2O2. The results show two isoenzyme for POD. This result was also obtained after precipitation with ammonium sulphate. Ammonium sulphate was slowly added to the homogenate stirred until complete dissolution. Then the mixture precipitate was dissolved in 1ml of phosphate buffer and dialyzed in a dialysis tube for 12h and then running on nondenaturing polyacrylamide gel The results showed two isoenzyme for POD again.

Keywords: peroxidase, isoenzyme, tea
Title:
Genetic basis of some prevalent Congenital Heart Disorders

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Abstract:
Heart disorders are one of the main issues of interest in medical societies and one of the main reasons of death in the world. Prevalence of Congenital Heart Disease (CHD) ranged from 4 to 10 per 1000 live births that 40% of them are diagnosed in the first year of life. The establishment of left-right asymmetry is very important to the normal development of heart. Secreted FGF, BMP, Nodal and Wnt act as input signal of symmetric cardiac morphogenesis: BMP2, FGF8, Shh/Ihh and Nodal function as positive regulators, where as Wnt is negative regulator. The cardiogenic genes: NKX2.5, SRF, GATA4, TBX5 and HAND2 compose the core regulatory network of cardiac morphogenesis, controlling heart looping, left-right symmetry and chambers formation. Mitral Valve Prolapse(MVP) is a relatively common condition with a prevalence ranging from 2.4% to 15% of the adult population. Two types of genes that interfering in heart valve development are Smad6, NFATc1. NFATs are transcriptional factors. In the nucleus, the NFATc combine with NFATn to produce NFAT active transcriptional complexes. The requirement for NFAT activity in valvuloseptal development was first demonstrated in NFATc1 null embryos, such embryos die due to valvuloseptal defects including hypomorphic semilunar and atrioventricular valves, as well as ventricular septal defects. To date, 10 different Smad genes have been isolated categorized into three subgroups involved in development. Smad4 is a member of the SMAD proteins that binds to all pathway-specific SMADs. In vertebrates, Smad6 and Smad7 function as inhibitors of BMP and TGF-β signaling. In this review we summarized some important pathways involved in normal development of heart valves and their related disorders.

Keywords: Genetic Basis, Congenital Heart Disorders
Title:
The investigation of functional sequence and structural features in anticancer endostatin fragments

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Abstract:
Endostatin, a peptide derived from proteolysis of collagen XVIII, is an endogenous inhibitor of angiogenesis and tumor growth. Several antiangiogenic activities have been reported for this protein, such as inhibition of endothelial cell proliferation, migration, and tube formation. Endostatin also suppresses vascular endothelial growth factor (VEGF)–induced vascular permeability. Some of the researchers synthesize peptides that derived from this protein. Some of this peptides have antitumor activities and others not. The memorable point about peptides this is that some of the active peptides are more than active from the native endostatin. With attention this point we used molecular dynamics (MD) and molecular docking simulation methods and found functional sequence and structural features in this peptides.

Keywords: endostatin - sequence and structural features - molecular dynamics simulation - molecular docking
Title: Peroxidase isoenzyme from Grass species Festuca

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Abstract: Peroxidases (donor: H2O2 oxidoreductase, EC 1.11.1.7; POD) are glycoproteins with ubiquitous distribution in the plant kingdom, showing generally several isoenzyme forms, high thermo-resistance and activity regeneration after heat inactivation. The multiple isoperoxidase forms found within the same plant source can differ with respect to molecular mass, isoelectric point, pH and temperature optima. In this study we determined isoenzyme of POD from a sub-species of grass Festuca. Festuca which belongs to the grass family Poaceae, distribution ranges through much of the northern to central U.S. During cool season grasses remain green through the year, tolerate shade and resist drought. For determine to isoenzyme plant material was harvested directly into liquid nitrogen and 0.5 g of frozen tissue was ground in phosphate buffer, pH 7.8 and subsequently the slurry was centrifuged at 4 °C. Peroxidase activity was assayed spectrophotometrically in 470 nm using guaiacol as substrate. Total protein content was measured according to the Bradford method using bovine serum albumin as a standard. The number of isoenzyme and activity of POD determined using 12% non-denaturing polyacrylamide gel. Peroxidase bands were detected by immersing the gel in a solution of phosphate buffer containing guaiacol and H2O2. The results showed three isoenzyme for POD. This result was also obtained after precipitation with ammonium sulphate. Ammonium sulphate was slowly added to the homogenate stirred until complete dissolution. Then the mixture precipitate was dissolved in 1 ml of phosphate buffer and dialyzed in a dialysis tube for 12 h and then running on non-denaturing polyacrylamide gel. The results showed three isoenzyme for POD again.

Keywords: Peroxidase, Festuca, I soenzyme
Title:
The structural changes in human hemoglobin upon interaction with a new designed Pd (II) complex

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Abstract:
Anticancer drugs based on heavy metals are believed to induce apoptosis in cancer cells by covalently binding to DNA. Some anticancer drugs can bind to hemoglobin (Hb). The interaction of these drugs with Hb may be caused by changes in the tertiary structure of this protein and so, equilibrium between two structures, R and T, are to the right or left, resulting in the tendency of Hb for oxygen decreases or increases. In this study, we have investigated the interaction of a new synthesized anti-cancer compound 1,10-phenanthroline hexyl dithiocarbamato palladium (II) nitrate) with Hb at two different temperatures of 25 and 37 °C by fluorescence and circular dichroism (CD) spectroscopic methods. Fluorescence data revealed that the Pd (II) complex is able to quench the intrinsic fluorescence of Hb. The values of $\Delta H^\circ$, $\Delta S^\circ$, and $\Delta G^\circ$ indicated that the van der waals force or hydrogen bond interactions might play a major role in the interaction of complex with Hb. The far-UV-CD studies display that the regular secondary structure of Hb had no significant changes. The results obtained to this study suggested that interaction of this complex with Hb may be important to improved understanding of side effects of new synthesized drugs on their carriers.

Keywords: Hemoglobin, Palladium (II) complex, Fluorescence, Van der waals force, Hydrogen bond.
Title:
Tetra-primer ARMS PCR for detection of A934C ABCC4 genotype in children with Acute Lymphoblastic Leukemia

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Abstract:
Therapy resistance in a significant number of children with Acute Lymphoblastic Leukemia (ALL) is still a major difficulty to successful treatment. The A allele of C934A is associated with reduced event-free survival and increased risk of relapse. In this study the genotype of A934C ABCC4 will be appear by tetra-primer ARMS PCR in Iranian children with ALL. Tetra-primer ARMS PCR is a simple, effective and economical SNP genotyping method based on Allele Specific (AS) primers. Four primers are required to amplify a larger fragment from template DNA containing the SNP and two smaller fragments representing each of the two AS products. Primers are designed in such a way that the two allelic products differ in size and can be separated by agarose gel electrophoresis. To enhance the specificity of the reaction, in addition to the first mismatch at the 3' end of AS primers, an extra mismatch is also deliberately introduced at the third position from the 3' end of each of the two inner AS primers. Four primers, one pair of inner AS primers and one pair of outer standard primers, are required in a single PCR reaction. The length of AS product for A allele is 236 bp and for C allele is 135 bp. in addition product of two outer standard primers is 324 bp. The process in ongoing and collecting of data has not been finished yet.

Keywords: Acute Lymphoblastic Leukemia, Relapse, SNP genotyping, Tetra primer ARMS PCR
Optimization of acteoside extraction method from Scrophularia striata cell culture

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Abstract:
Introduction: Suspension culture of Scrophularia striata Boiss. (Scrophulariaceae) accumulate acteoside (an anticancer agent) and could therefore serve as an alternative source of this important phenylethanoid glycoside. The present work compared several acteoside extraction methods and optimized the best one by using single factor experiments.

Method: S. striata cell culture was established from in vitro propagated plantlets and subcultured in MS medium supplemented with 0.5 mg/L NAA + 2 mg/L BA every 15–17 days. Acteoside extraction methods were assayed and the best one was optimized by single factor experiments, studying the effect of ethanol and methanol concentrations, extraction time, shaking and sonication time. Also, a high performance liquid chromatographic method was established for the determination of acteoside in the samples. The extracts were analyzed on a C18 column using a mixture of CH3OH - 0.4% aqueous HAc as the mobile phase with UV detection at 333 nm.

Results: Cell culture accumulated enough acteoside to be analyzed by HPLC. The results showed that acteoside can be extracted more efficiently with 90% methanol than with distilled water or methanol. Extraction time, shaking and sonication time were not effective for extracting acteoside. Therefore, S. striata were extracted with 90% methanol.

Conclusions: The optimized method based on 90% methanol extraction combined with HPLC quantification was able to determine small amounts of acteoside in S. striata cell culture, showing that this system could constitute a possible alternative source of acteoside to wild plant.

Keywords: Cell culture; extraction; HPLC; Scrophularia striata; acteoside
Title: Modified electrodes for biofuel cell

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Abstract:
Quaternary ammonium bromide salt-treated Nafion membranes are an ideal environment for enzyme immobilization. Because these quaternary ammonium bromide salt-treated Nafion membranes increase the mass transport of ions and neutral species through the membrane, they are also ideal for modifying electrodes. Therefore, high current density bioanodes and biocathodes are formed from poly (methylene green) (an electrocatalyst for NADH) modified electrodes that have been coated with a layer of tetrabutylammonium bromide salt-treated Nafion with dehydrogenase and Laccase enzymes immobilized within the layer. Ethanol/O2 biofuel cells employing these bioelectrods have yielded power densities of 1.69 mW/cm2 with a single-enzyme system (alcohol dehydrogenase) and cell voltage of 0.21 V.

Keywords: biofuel cell; Enzyme, Laccase, alcohol dehydrogenase, Nafion, poly methylene green.
Title:
Screening enzymatic and nonenzymatic antioxidant activity of tea leaves harvested from various planting heights.

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Abstract:
Tea extracts are powerful antioxidants due to the presence of chemical compounds such as epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC) and epicatechin (EC) (1). Most of these compounds act as effective scavengers of free radicals (2). It has been shown that tea, the common drink worldwide, is able to reduce risk of coronary heart disease in aged men (3). Tea polyphenols are considered to be responsible for the anticarcinogenic, antimutagenic and protection against cardiovascular diseases of tea (4). However, the composition of various chemical compounds in tea may differ depending on growth conditions and region of production (5). In this study, the crude extracts of tea collected from different heights of Lahijan mountains were compared for their enzymatic and nonenzymatic antioxidant capacity.

In practice, leaves of tea were harvested from the territory of Lahijan (534m, 305m and 50m). To make crude extracts from tea leaves, potassium phosphate buffer 50mM (PH 7) containing EDTA 0.5mM was used. Crude extracts were used for Superoxide dismutase (SOD; EC 1.15.1.1) and peroxidase assay. Nonenzymatic antioxidant activity was measured by DPPH radical scavenging assay, Folin–Ciocalteu assay and FRAP assay.

As result, a considerable difference in activity of SOD and POD and nonenzymatic antioxidant activity tests between the tea leaves that harvested from various planting heights, was observed.

Keywords: antioxidant, tea, free radicals, disease, enzyme.
Title: Evaluation of antigenotoxic effects of Glucosamine and N-acetylglucosamine on human peripheral lymphocytes exposed to oxidative stress

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Abstract: It is becoming increasingly evident that oxidative stresses and DNA damage are related to various diseases and pathological conditions such as carcinogenesis, atherosclerosis, and aging and so antioxidants can minimize their incidence. D-Glucosamine (GlcN) is a naturally occurring amino sugar that possesses excellent antioxidant activities. The purpose of this study was to evaluate the antigenotoxic effects of D-Glucosamine (GlcN) and its acetylated analogue (N-acetylglucosamine: GlcNAc) on human peripheral lymphocytes using alkaline single cell gel electrophoresis approach (comet assay).

Method: Lymphocytes were isolated from blood samples taken from healthy volunteers. DNA breaks and resistance to H2O2-induced damage were measured using comet assay. Human lymphocytes were incubated with GlcN and/or GlcNAc (2.5, 5, 10, 20 and 40 mM) alone or a combination of different concentrations of GlcN and and/or GlcNAc and H2O2. Untreated cells, H2O2 (25 µM) and were considered as negative control and positive control for our study, respectively. Single cells were analyzed with “TriTek Cometscore version 1.5” software. The DNA damage was expressed as percent tail DNA.

Results: Glucosamine exhibited a concentration dependent increase in protection activity against DNA damage induced by 25 µM H2O2 (from 38% to 5%) but its acetylated analogue (GlcNac) shows very weak protection activity only at highest concentration (40 mM).

Conclusions: Our results indicated that glucosamine could be a suitable agent for preventing chemically induced DNA and chromosome damage in vitro. However, further studies should be performed to better understand the mechanisms and conditions underlying its chemopreventive activity.

Keywords: Antigenotoxicity, Comet assay, Glucosamine, N-acetylglucosamine
Title:
Design of HIV electrochemical immunosensor using gold nanoparticles and secondary labeled antibody

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Abstract:

Introduction: The human immune deficiency virus (HIV) caused Acquired immune deficiency syndrome (AIDS) is an acute communicable immune deficiency disease. A very important method for controlling AIDS is the analysis laboratory diagnosis of HIV infection. Since the p24 antigen, the HIV-1 capsid protein, is detectable several days earlier than host-generated HIV antibodies following HIV exposure, it is a remarkable diagnostic interest. In this research, a modified glassy carbon electrode was designed for sandwich HIV-1 p24 immunosensor.

Methods: A modified gold nanoparticles-modified electrode (GNP/LC/GCE) was utilized. This electrode was created by L-Cysteine-modified glassy carbon electrode that gold nanoparticles assembly by themselves on the surface of it and secondary antibody labeled with Anthraquinon-2-carboxylic acid. The GCE electrode was scanned in the potential range from −1.0 to 1.5V at 0.10Vs−1 for 10 cycles in 0.1 mol L−1 PBS containing 5.0×10−3 mol L−1 L-cysteine. Subsequently, the treated GCE was rinsed thoroughly with double-distilled water and immersed in colloidal gold solution for 10 h at 4 °C to fabricate the gold nanoparticles self-assembled GCE.

Results: An oxidation peak for anodic immobilization was observed at 1.30V in the first anodic scan, and another redox peak was observed at -0.32V in the reverse scan.

Conclusions: This electrode may be applied to immunosensor for clinical samples, being distinguished by its ease of use and reproducibility.

Keywords: p24 antigen, HIV, Sandwich amperometric immunosensor, Anthraquinon-2-carboxylic acid
Title:
Involvement of lipoxygenase in ultrasound-stimulated Taxol biosynthesis in suspension cultured Corylus avellana cells

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Abstract:
Elicitation is based on signal (elicitor) induced expression of defense related genes, which subsequently results in increased synthesis of secondary metabolites in intact plants or in plant cell cultures. Studies in recent years demonstrated that lipoxygenase (LOX) might play an important role in the plant defence response. In the present work we studied the role of LOX in Taxol biosynthesis pathway in Corylus avellana suspension cultures under ultrasound elicitor. The cells were grown in LS media and were exposed to ultrasound at power density of 4 mW/cm2 for 4 to 40 min. Changes of LOX activity, expression of 1-Deoxy-D-Xylulose-5-Phosphate Reductoisomerase (DXR) and phenylalanine ammonialyase (PAL) were measured. LOX activity rapidly began to increase after sonication, attained its peak at 48 h. No change was observed in LOX activity of the control cultures. Significant increase also was observed in the expression PAL and DXR genes. The results demonstrated that LOX is involved in the elicitor induced production of Taxol.

Keywords: lipoxygenase, phenylalanine ammonialyase, Taxol, ultrasound
Title:
Total protein and catalase (CAT) activity in chenopodium ambrosioides

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Abstract:
Chenopodium ambrosioides is an herb which belongs to chenopodiaceae family. It has been used as an anthelmintic (medicine for controlling internal parasites) for many years. This study catalase, a principle antioxidant enzyme from chenopodium ambrosioides. Catalase (CAT: EC 1.11.16) is a enzyme to have present in structure of plant cells. This enzyme is widely distributed in a variety of life forms, including animals, plants, microbes and usually only absent from strictly anaerobic organisms. All aerobic organisms during the course of metabolism form reactive oxygen species (ROS) as by products. Superoxide (O2•−), nitric oxide (•NO), hydroxyl ion radicals (OH) and hydrogen peroxide (H2O2) are the common ROS. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. The leaves were homogenized in an ice cold mortar using 50 mM sodium phosphate buffer, pH 7.0, 1% polyvinylpyrrolidone and 1 mM EDTA finally was used to determine enzyme activities. Catalase activity was determined by following the consumption of H2O2 at 240 nm 90 s. The assay mixture containing 100 mM potassium phosphate buffer (pH 7.0), 15 mM H2O2 and 50 µl leaf extract in a 3 ml volume. Unit was defined as µmol H2O2 decomposed per 1 min. Total protein content was measured according to the Bradford method using bovine serum albumin (BSA) as standard. The number of isoenzyme and activity of CAT determined using 8% nondenaturing polyacrylamide gel. The results showed a high activity and only one isoenzyme for POD.

Keywords: Chenopodium ambrosioides, catalase, purification, oxidative stress
Title:
Prediction the binding capacity of tryptic peptides of betalactoglobulin to anticancer drugs using bioinformatic approach

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Abstract:
Introduction Betalactoglobulin (BLG) is an abundant whey protein in milk of ruminants and the core member of lipocalin family. The BLG structure as determined by X-ray shows a typical β-barrel fold, characterized by eight antiparallel β-strand, an α-helix and a short C-terminal helix that enwrap a central calyx. BLG is a carrier of small hydrophobic molecules, including retinoic acid. Acidic resistant, abundance in cheap protein sources, easy purification and structural features make BLG a potential carrier for various drugs. The aim of this study is to determine the potential tryptic peptides of BLG in order to study their binding to three anticancer drugs using molegro software.

Method Crystallographic structure of native BLG obtained from Protein Data Bank. The structure of 4 peptides used in this study was constructed using Arguslab software. Docking analysis was performed between tryptic peptides and anticancer drugs including Adriamycin, Captothecin and Fluorouracil using Molegro Virtual Docker. Moldock score was determined in each cases.

Result Moldock score indicated that a tryptic peptide (Val 15 - Arg 40) from native BLG has most affinity to bind drugs. It can bind to drugs with moldock score -106.413, -93.2222 and -43.5102 respectively. This peptide can bind to drugs through Ala25, Ala26, Asp28, Asp33, Ile29, Leu31, Leu32 and Ser27 amino acids.

Conclusion Naturally derived peptides would be expected to have no side effects in comparison with intact protein. Our study explores structural specificity of the milk derived peptides and valuable insight in designing highly specific carrier for drugs.

Keywords: Betalactoglobulin (BLG), Tryptic peptide, anticancer drug, Molegro Virtual docker
Title:
fluorescence spectroscopic studies of the interaction between nanoemulsion including drug with human Hemoglobin

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Abstract:
(Hb) is one of important and effective of blood proteins. In the present study, the interaction of a new synthesized nanoemulsion (as a new drug carrier) with human Hb, as a model protein, was investigated by different spectroscopic methods of fluorescence at different temperatures of 25, 37, 42 and 47 0C. Intrinsic fluorescence studies show that nanoemulsion have ability to quenching of fluorescence intensity Hb via static quenching mechanism. Also, in the presence of different concentrations of nanoemulsion, the maximum emission wavelength of Hb was shifted to a smaller wavelength (blue shift), which indicates that the hyrophobicity of Trp environment was decreased by adding nanoemulsion. Also, the binding site of nanoemulsion may be in the near of tryptophan residue. From above results, it can be concluded that our new designed nanoemulsion can change the secondary and tertiary structure of Hb at different temperatures.

Keywords: Hemoglobin, nanoemulsion, fluorescence
Title:
Numerical Analysis of the Sternal Closure in the Open Heart Surgery for the Finite Element Model of a Complete Human Chest

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Abstract:

Introduction: Median sternotomy has been extensively used by cardiothoracic surgeons since 1957 in order to gain access to the heart since it provides an excellent exposure. Sternal closure is the last step of median sternotomy. Different techniques of sternal closure have been described but the optimal technique of median sternotomy remains controversial. The objective of this study was to analyze the structural response of the wires and separated sternum during this procedure using finite element methods.

Material and methods: Two-dimensional thoracic computer tomography scans (1.5-mm slices) were segmented and analyzed by image processing techniques and transferred into a three-dimensional finite element model of a complete chest. Linear elastic law models were used for several regions. Then, the sternal closure process on the sternum was modeled and a basic model of the lungs was used for applying intrathoracic loads.

Nonlinear contact conditions were applied between the two Sternal parts and between fixation wires and sternum. The structural response of this model was investigated under normal breathing and a severe cough by means of lung model.

Result and conclusion: The results show that the lower regions of the sternum and also lateral sides of sternum in contact with wires are the regions which have maximum stress values therefore the risk of failure or damage in these areas is high. The maximum stress for a severe cough load case is 243 N/mm² for the wires and 65.6 N/mm² for sternum bone that can lead to failure in this region for more severe cases.

Keywords: biomechanics, sternotomy, sternal closure, finite element method, sternum, wire
Title: Interaction of LMG160 ribonucleoprotein particle with DNA in solution

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Abstract: Low mobility group (LMG) proteins which are extracted from eukaryotic chromatin in low ionic strength, have a high molecular weight. In this laboratory, a fraction of these proteins was isolated and purified from rat liver nuclei, named LMG_{160}. It has been found that LMG_{160} is a ribonucleoprotein particle (RNP) with an inhibitory effect on in vitro transcription system. In the present study, the interaction of intact (I-LMG_{160}) and RNase-treated (T-LMG_{160}) LMG_{160} with rat liver DNA molecule in different I/T-LMG_{160}:DNA ratios (w/w) was investigated employing thermal denaturation. The results showed that, DNA thermal stability was increased by increasing I-LMG_{160}:DNA ratio (w/w) whereas, increasing T-LMG_{160} concentration had no significant effect on DNA melting point (T_m). The estimation of thermodynamic parameters revealed that the ΔH_m and ΔS_m values of DNA unfolding, raised in the presence of I-LMG_{160} but in the case of T-LMG_{160} there was no remarkable changes in these parameters. It is concluded that, only intact form of LMG_{160} is able to interact with DNA molecule but RNase-treated form is not. Probably the presence of RNA part in LMG_{160} is required for the suitable DNA:LMG_{160} interaction through DNA:RNA linkage. It seems that, I-LMG_{160} inhibitory effect on in vitro transcription system is achieved as a result of DNA compaction, meanwhile T-LMG_{160} which has no effect on in vitro transcription system, doesn’t interact with DNA molecule. Therefore RNA moiety plays the main role in the LMG_{160} ribonucleoprotein particle function.

Keywords: ribonucleoprotein, low mobility group (LMG) proteins, LMG160, thermal denaturation, soluble DNA
Title:
spectroscopic study of topotecan binding to chromatin

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Abstract:
Topotecan (TPT) is in clinical use as an antitumor agent. It acts by binding to the covalent complex formed between nicked DNA and topoisomerase I, and inserts itself into the single-strand nick, thereby inhibiting the religation of the nick and acting as a poison. In this study, we have investigated the interaction of topotecan with chromatin in solution to elucidate the mechanism of its action, employing fluorescence spectroscopy technique. Chromatin, in the absence of the drug, at 278 nm exhibited a peak with a maximum at 334 nm. Addition of topotecan to chromatin solution decreased the fluorescence emission intensity without any red shift in the emission maxima (Imax) as the drug concentration was increased. As drug concentration is increased, the binding affinity is increased and reaches to a maxima (at 40 µg/ml of drug) providing saturation state of the binding. Quenching of TPT with phenylalanine residues of the protein components of the chromatin, employs that the histone proteins may play a role in TPT-chromatin interaction process. The results clearly suggest that apart from topoisomerase I, chromatin can be considered as a new target for this drug which opens a new insight into its anticancer activity in tumor cells. Topotecan interacts with chromatin components and probably its binding occurs through both interaction with chromatin components and intercalation into base pairs of DNA.

Keywords: Topotecan, chromatin, topoisomerase I, fluorescence spectroscopy
Title:
stable expression of CCX-CKR receptor in HEK293T cells

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Abstract:
Introduction: Chemokines act through their classical receptors (G protein coupled receptors) in downstream cell signaling pathways. They have important roles in vivo by directing the migration of immune cells, organ development, angiogenesis, and etc. Chemokines are vital for tumor progression; they take part in growth, angiogenesis, metastasis, and tumor cell survival in cancer. In the last years, atypical chemokine receptors, as a decoy receptors, with high affinity for chemokines, have been investigated. Because the DRY motif modification in this receptors, they couldn't interact with G proteins and as a consequence, no intracellular signaling was observed. So, they can compete with signaling receptors, degrade chemokines, and change the localization of chemokines. CCX-CKR receptor is a scavenger of CCL19, CCL21, CCL25, and CXCL13 chemokines. This chemokines and their typical receptors (CCR7/CCL19 (CCL21), CCR9/CCL25, and CXCR5/CXCL13) are involved in cancer growth and metastasis. As a regulator of chemokine level, this receptor should be considered in cancer, and for therapeutic aims.

Method: CCX-CKR expression constructs were made in p3XFLAG-Myc-CMV™-26 Expression Vector and were used to generate stable transfectants in human embryonic kidney 293 (HEK293) cells. Results: we constructed p3XFLAG-Myc-CMV™-26 Expression Vector encoding CCX-CKR. This allowed for detection and selection, using an anti-FLAG Ab (DYKDDDDK Tag Antibody), of the most highly expressing stable transfectants. Conclusions: HEK cells have been extensively used for functional analysis of chemokine receptors. HEK293 cells stably expressing the FLAG epitope-tagged CCX-CKR were selected for further analysis.

Keywords: Stable expression, CCX-CKR receptor, chemokines
Title: Exogenous DNA internalisation by chicken sperm cells

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Abstract: From 1971, several reports made the claim that sperm cells could associate with exogenous DNA molecules (transgene) and transfer these molecules during fertilization. Sperm ability to take up transgenes can be exploited to transmit novel genetic information to the offspring after fertilization. The present attempt explore the possibility of transfecting chicken spermatozoa with exogenous DNA. For improving the efficiency of DNA uptake by sperm cells we combine sperm-mediated gene transfer (SMGT) with restriction enzyme-mediated integration (REMI) and lipofection. also the sperm cells are treated with Triton- X or subjected to freeze-thaw cycles to cause partial disruption of the sperm membrane which could facilitate uptake of transgenes. Data demonstrate that lipofection of plasmid DNA with restriction enzyme is a highly efficient method for the production of transfected sperm to produce transgenic offspring. SMGT may be able to provide efficient, rapid and low-cost protocols for animal transgenesis but stable modifications of the genome are difficult to detect. The size of the exogenous DNA did not seem to influence the internalisation efficiency of chicken spermatozoa but for further studies we can investigate the effect of size of exogenous DNA.

Keywords: sperm, chicken, REMI, lipofection.
Title:
Studies on the binding of epirubicine as an anticancer drug on chromatin

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Abstract:
Introduction: Epirubicine is an anthracycline anticancer drug widely used in chemotherapy. It acts by intercalating with DNA base pairing and triggering DNA cleavage via topoisomerase II, resulting in death of cancer cells. The binding of epirubicine to DNA has been studied in detail, however, in the cell nucleus, DNA is compacted into a complex structure built from the interaction of histones with DNA as the name of chromatin.Methods: In the present study we have investigated the effect of epirubicin on chromatin by employing UV/Vis, fluorescence and circular dichroism spectroscopy.Results: The results shown that addition of various concentration of epirubicine to chromatin precipitated it in a dose dependent manner and increase absorbance at 480nm. chromatin exhibits an emission spectrum in the position corresponding to tyrosine with a maximum intensity at 305nm after excitation at 278nm. Upon addition of epirubicine, fluorescence emission intensity of chromatin was decreased in a dose dependent manner. Fluorescence data revealed that the fluorescence quenching of epirubicne was the result of the formed complex of epirubicine-chromatin, and the binding constant(Ka) and binding number obtained is 1.8 x 10 4 at 298 K and 4, respectively for the primary binding site. Circular dichroism spectra showed the induced conformational changes in chromatin by the binding of epirubicine.Conclusion:from these results it is concluded that Epirubicine showed higher affinity to chromatin than DNA.

Keywords: Chromatin, Epirubicine
Title:
Compare of Podophyllotoxin content in cell culture in different medium of endemic Linum persicum

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Abstract:
*Linum persicum* is an endemic plant growing wild in Iran which have considerable amounts of lignans. These lignans are used to produce anticancer drugs such as, etoposide, etophose and teniopside [1]. In this study we investigated podophyllotoxin content in cell culture in 16 phytohormon treatment. Podophyllotoxin content was higher in 1 mg L\(^{-1}\) naphthaleneacetic acid/ 0.4 mg L\(^{-1}\) kinetin treatment. Amount of podophyllotoxin in this treatment was 0.05 mg/gr(FW).

Keywords: Lignans, Linum persicum, Podophylotoxin.
Title: 
Baneh extract induces peri-nuclear localization of Livin protein and decrease in Livin mRNA expression in breast and colorectal cancer cells

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Abstract: 
Introduction: Livin/ML-IAP is a member of the Inhibitor of apoptosis proteins (IAPs) family that negatively regulates apoptosis. In addition, its elevated expression in human malignancies makes it an attractive target for new drug development in cancer therapy. We have previously reported that the pericarp methanolic extract of *Pistacia atlantica sub kurdica* (*Baneh*), a potent and novel natural anticancer agent, induces apoptosis in human breast T47D and colorectal HT29 cancer cells via a series of events including DNA fragmentation, phosphatidylserine translocation and caspase-3 activation. Methods: In this study, we evaluated the expression of Livin mRNA and protein following treatment with *Baneh* extract and doxorubicin (Dox) in HT29 and T47D cancer cell lines in comparison to the Dox by real-time RT-PCR and Immunocytochemistry (ICC) techniques. Results: The qRT-PCR results showed considerable reduction in expression of mRNA level for Livin in *Baneh*-treated T47D and HT29 cancer cells. In addition, the ICC data indicated that in non-treated HT29 and T47D cells, high Livin expression was detectable that localized to the cytoplasm and nucleus. But the *Baneh*-treated T47D and HT29 cancer cells showed reduced Livin expression and peri-nuclear localization. Conclusion: These data demonstrate for the first time that down-regulation of mRNA and protein expression of Livin are other mechanisms of action of *Baneh* extract for its antitumor activity. Therefore, *Baneh* extract could be considered for further evaluation in cancer therapy as anti-Livin candidate compound.

Keywords: Baneh extract, IAP, Livin, HT29, T47D
Title: The Effect of Calcium on alleviation of Cadmium toxicity on superoxide dismutase activity in Matricaria chamomilla L. plants

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Abstract:

Introduction: Heavy metal pollution of environment is one of the most important ecological problems on world scales. Heavy metals exert to plants inhibition their growth and productivity. Also, these metals are absorbed and accumulate by plants. Cadmium is one of the most contaminant metals in environment which has several effects on plant anatomical and morphological characters. Ca can decrease the uptake, translocation and accumulation of Cd in Plants. application of moderate amount of exogenous Ca can ameliorate heavy metal toxicity through competition for metal ion influx. Matricaria chamomilla is an important medicinal plant. In this research The effect of calcium on the alleviation of cadmium toxicity on the activity of superoxide dismutase enzyme of Matricaria chamomilla was studied.

Method: M. chamomilla seedlings treated with 0, 0.1, 1, and 5 mM Ca, under 0, 120, and 180 µM Cd conditions, respectively in hydroponic culture. Treatment were harvest after one week.

Results: The result shown that when plants were simultaneously exposed to Cd and Ca, Cd toxicity was decreased.

Conclusions: The result indicated that superoxide dismutase activity have decreased with increase in Ca concentration in the roots and shoots of Cd-stressed plants.

Keywords: Cadmium, Calcium, Matricaria chamomilla, superoxide dismutase
Title: Ethical Challenges of Human Life Span Using Molecular Modification

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Abstract: Introduction: Aging and then death is a universal process that began with the origination of life during long centuries but recent advances in human genetics suggest that it may become possible to genetically manipulate telomere and telomerase system to alter the mechanisms of aging and extend the human life span. Method: There are biological and moral reasons to carefully consider the implications of exploiting this technology. For example, concerning the nature and implications of the value of life, as well as, and as distinct from the value of life extension should be mentioned. Also economic disincentives, disease-specific lobby groups, scientific careerism, and ineffective nostrums, together with gerontologiphobia, must be overcome before such research can improve human life frame. Also prolonging life only makes sense if quality of life is maintained. Results: Significantly human life span would also raise a large number of unprecedented individual and social problems: Would we really want to live to several century? Is such a goal ethical? What would this putative longevity do to our present social structures and arrangements? Would we get a better society or a worse one? Alongside of above possible problems human life span include huge increases in the number of old and very old persons, the likelihood of a massive increase in health expenditures for the population aged old and older, the potential for outliving financial resources, challenges to the viability of social security and pensions, concerns about quality of life, and possible intergenerational antagonisms. Some other problems of human life span included: changing the patterns of work life and labor-force participation, necessarily changing the interfamily care giving patterns, changing the level of disability and dependence of older people and so on. Conclusions: A life span significantly longer than the present norm would be undesirable because it would severely weaken the connections between past- and future-oriented mental states and turn the psychological grounds for personal identity and prudential concern for our future selves. In addition, the collective effects of longer lives might lower the quality of life for all people.

Keywords: Life Span, Molecular Modification, Ethical Challenges
Title:
Impairment of social behavior in the initial stages of Alzheimer’s disease is caused by increased instinctive anxiety

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Abstract:

Introduction: Alzheimer’s disease (AD) is a progressive neurodegenerative disease which results in cognitive impairment. Objectives: This study aimed to investigate the alterations of instinctive anxiety level and its effect on social learning in AD. Methods: AlCl$_3$-induced mouse model of AD was used which was developed in ten months old mice by intraperitoneally administering AlCl$_3$ (150mg/kg/day) for 14 days. Results: On day 14, treated mice were subjected to different behavioral tests including elevated plus maze (EPM), social novelty and tube dominance. Instinctive anxiety level and exploratory behavior was assessed using EPM. Time spent in closed arms by AD mice was significantly higher (97.53±0.83, n=10) compared to control mice (89.80±1.81, n=10) indicating increased instinctive anxiety (p=0.001). AD mice showed reduced exploratory activity (p=0.002) by showing less number of entries (3.00±0.83, n=10) into open arms compared to control (10.20±1.88, n=10). Social novelty test showed a significant deficit (p=0.0003) in preference for social novelty in AD mice (49.90±21.11, n=10) compared to control (188.10±22.67, n=10). AD mice showed impaired sociability during exposure to an unfamiliar conspecific (p=0.0019). Tube dominance test was performed to assess the aggressive tendencies and social dominance, however, no significant difference (p>0.05) in aggressive tendencies of AD mice (66.40±11.13, n=10) compared to control (53.00±10.18, n=10) was observed. Conclusion: These data showed that AlCl$_3$-induced mouse model demonstrated, primarily, amygdala and hippocampus dependent cognitive impairment in the initial stages of AD, while sparing aggressiveness. Therefore, this model can be used for the drug testing and diagnostic studies in future.

Keywords: Social behavior, learning, Alzheimer’s disease, amygdala
Title:
Combination of Spectroscopic and Electrochemical Methods for Discerning the Effect of Osmolytes on Biological Activity and Electron Transferring in Catalase

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Abstract:
Catalase as a prominent homotetramer, catalyses the disproportionation of $\text{H}_2\text{O}_2$ into water and molecular oxygen. It has broad applications in various sectors of industry and medicine. Crucial to those roles are the heme prosthetic groups. Osmolytes are organic compounds that affect the osmotic pressure and are categorized into compatible and non-compatible ones. The former category maintains and/or enhances the biological activity and does not perturb structural stability of catalase, whereas the latter hinders the biological activity and destabilizes the protein to various degrees. Biological activity was assessed by the decrease in the absorbance of hydrogen peroxide monitored at 240 nm according to the method of Aebi. The electron transfer was monitored by redox titration, and coupled reaction using xanthine oxidase in addition to the electrochemical techniques. In the redox titration, the mixture of catalase and potassium ferricyanide was reduced by potassium ferrocyanide. In the coupled reaction, the xanthine/xanthine oxidase couple and a redox indicator were used. In both cases midpoint redox potential ($E_m$) was recorded for the native catalase and for the catalase interacted with proline and histidine. In the electrochemical section a catalase-modified glassy carbon (GC) electrode was constructed using amine-functionalized multi-wall carbon nanotubes (MWCNT) and a room temperature ionic liquid (RTIL). Peak cathodic currents ($I_{pc}$) were measured as the indicator of electron transferring. The average of cathodic and anodic peak currents, served as the formal potential ($E^\circ$). $E_m$ was confirmed using the y-intercept in the log [ferrocatalase]/[ferricatalase] versus log [ferricyanide]/[ferrocyanide] plots. Proline increased $E_m$ (decreased $DG_{ET}$) whereas histidine increased it (made $DG_{ET}$ less negative). Likewise the y-intercept of log [DCIPox]/[DCIPred] versus log [Catalase ox]/[Catalase red] in the xanthine/xanthine oxidase coupled reaction, and the electrochemical investigations demonstrated congruent results, bearing testimony to more favorable free energy of ET in the presence of proline compared to that of native, and in contrast to retarded ET caused by interaction with histidine. The more facile electron transferring in the presence of proline is also depicted in higher values of $I_{pc}$ compared to that of native. Lower values of $I_{pc}$ in the presence of histidine allude to retarded electron transfer. Metalloproteins play an extremely pivotal role in the living organisms, reflected by the effort invested in dissecting the biological activity and electron transferring characteristics. Combined studies however that try to correlate those characteristics are hardly undertaken. In the current study the effects of osmolytes on the electron transfer of catalase were investigated. The results confirmed that proline as a compatible osmolyte increases ET, whereas the electron transfer in the presence of histidine as a non-compatible one is retarded.

Keywords: Catalase, Proline, Histidine, Biological activity, Electron transferring
Title:
Probing of the Interaction Between Human Serum Albumin and A New Synthesized Pd(II) Complex Using Spectroscopic methods

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Abstract:
Human serum albumin (HSA) is an abundant, multifunctional and nonglycosylated, negatively charged plasma protein, with ascribed ligand-binding and transport properties, antioxidant functions, and enzymatic activities. In the present study, the interaction and side effects of a new designed anti cancer compound (1,10-phenanthroline butyl dithiocarbamato palladium(II) nitrate) on HSA have been investigated by different spectroscopic (UV-visible, fluorescence and circular dichroism (CD)) techniques at two temperatures of 25 and 37°C. By the analysis of fluorescence spectra, it was observed that this complex has an ability to quench the intrinsic fluorescence of HSA through a static quenching procedure. The number of binding sites and the association binding constants of Pd(II) complex were calculated at 25 and 37°C. Also, the negative ΔH° and positive ΔS° values resulted interaction of Pd(II) complex, using the vant’s Hoff equation, showed that the electrostatic interaction has a major role in the binding process. The quantitative analysis of CD spectra represented that Pd(II) complex induces significantly alterations in the secondary structure of protein via decreasing in the content of α helical structure of the protein. Our results suggest that the new synthesized Pd(II) complex can bind to blood carrier protein of HSA and change the tertiary and secondary structure of the protein, which may be considered as side effects of this new synthesized drug.

Keywords: HSA, Pd(II) complex, side effect, fluorescence, circular dichroism
Title:
Study of the interaction of oxaliplatin with chromatin protein: Histone H1

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Abstract:
Oxaliplatin, L-OHP is a third generation platinum antitumor analog in which 1,2 diaminocyclohexane (DACH) ligand substitutes for the amine groups of cisplatin. This drug exerts its cytotoxic effect mostly through DNA damage and forms intra and interstrand crosslinks in cellular DNA. Histone H1 is a very lysine-rich histone fraction of histone proteins which binds to linker DNA between adjacent nucleosomes to facilitate the folding of the chromatin fiber. In the present study we have investigated the interaction of oxaliplatin with histone H1 in solution using UV/Vis and fluorescence spectroscopy techniques. Histone H1 was isolated from calf thymus and purified. The protein was incubated in the presence and absence of various concentrations of the drug for an hour and then spectrophotometric measurements were carried out at room temperature. The results showed that upon addition of oxaliplatin, absorbance of histone H1 at 210nm was significantly decreased. The fluorescence emission intensity of histone H1 in the absence of oxaliplatin exhibit a characteristic fluorescence emission intensity maximum at 305nm corresponding to the maximum fluorescence emission intensity of tyrosine. Addition of increasing amount of oxaliplatin to histone H1 solution at a constant protein concentration resulted in a reduction of its emission intensity because of fluorescence quenching. Stern-volmer curve shown positive and linear relationship. From the result presented above it is concluded that histone H1 can be considered as potent target for this drug which open a new insight into mechanism of oxaliplatin action.

Keywords: oxaliplatin, histone H1, spectroscopy
Title: Quantitative Sequence-Activity Modeling of Linear Hexapeptide Antibiotics: An Approach for the Prediction and Description of Antifungal Activity

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Abstract: The growing emergence of bacterial/fungal superbugs in clinics has limited effectiveness of current antibiotics. Therefore, design and development of antibiotics with novel mode of actions represent a particular challenge. Antimicrobial peptides (AMPs) which act mostly by damaging bacterial/fungal cell membrane are effective molecules in innate immune system and can provide promising antibiotics for treatment of superbugs-associated infections. However, design of new AMPs with improved therapeutic index is always demanding. Quantitative-sequence-activity modeling (QSAM) is an effective chemoinformatic technique employing quantitative structure–activity relationship for biomolecules. In this approach, biosequence activities of therapeutic agents; e.g., peptides, are linked to functional/structural properties. Here, a QSAM study was performed on a series of linear hexa-AMPs with different activity profile against C. albicans superbugs using segmented principal component strategy. For this, structures of the amino acids in hexa-AMPs sequences were drawn and optimized by Hyperchem, v.8. Then, different structural information, such as constitutional, charges, and topological descriptors were extracted and classified into groups with similarity in their informational contents. Each group was separately subjected to principal component analysis (PCA). The extracted PCs were used as the descriptors of the model after variable selection. Our results showed that constructed models covered more than 85% of the variance in train and test sets. Also, information on the highlight zone of the hexapeptides (representing a general part of peptide structure with highest impact upon antifungal activity) was obtained. The applied descriptors for this structure–activity model were sensitive to polarity of amino acids in the peptide sequences. Acknowledgments: Financial support of this project by National Elites Foundation is highly appreciated.

Keywords: Antimicrobial Peptides, Superbugs, Chemoinformatic, Quantitative Sequence-Activity Modeling, Amino Acid Descriptors
Title:
The inhibitory effect of Aloin and camel peptides on human serum albumin glycation

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Abstract:
Reports from the Center for Disease Control and Prevention indicate that the incidence of diabetes mellitus is growing in the world. Diabetes mellitus, characterized by defective blood sugar regulation, occurs in two forms: type I and type II. The non enzymatic reactions between reducing sugars with amino groups of K residues of proteins is known as Maillard reaction. Advanced glycated end products (AGEs) are also formed in vivo, especially on long lived proteins such as HSA. In vivo AGEs formation contributes among others to the onset of diabetic complications, renal insufficiency and Alzheimers disease. Aloin is one of the main active phenolic components of Aloe vera. Aloin has received much recent attention and has been shown to be effective in treatment and prevention of various diseases such diabetes. Bioactive peptides derived from milk proteins have been shown to play many biologically important functions such as anti-cancer, antioxidant, anti-hypertensive. In this study the glycated human serum albumin (GHSA) was monitored in the presence of Aloin and bioactive peptides by fluorescence spectroscopy with 1-Anilinonaphthalene-8-sulfonate (ANS) method to study the tertiary structure of glycated HSA. The ANS is a compound that is used for probing the available hydrophobic domains in proteins. The ANS fluorescence spectra in the presence of protein samples were recorded over a wavelength range of 300–600 nm. The excitation wavelength was 385 nm. The highest hydrophobicity was observed for modified HSA incubated for 42 days with glucose. From the results of this it can be concluded that aloin and camel milk peptides reduced tertiary structure changes and glycation.

Keywords: Glycation, Bioactive peptides, Aloin, ANS fluorescence
Title:
ACE-inhibitory activity of peptide fractions of camel and bovine milk fermented by Lactobacillus fermentum PTCC1638

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Abstract:
Bioactive peptides release from milk proteins through digestive, microbial or plant enzymes or by fermentation using proteolytic microorganisms such as some lactic acid bacteria (LAB). The aim of this study was to compare the ACE-inhibitory of peptides fractions of fermented bovine and camel milk using Lactobacillus fermentum PTCC 1638. Bovine and camel milk samples were obtained from a commercial dairy farm in Tehran province and Gorgan province, Iran, respectively. Fresh whole bovine and camel milks were pasteurized at 80 °C for 20 min in water bath and cooled to 43 °C. Lactobacillus fermentum PTCC 1638 was then inoculated into milk samples and incubated at 37 °C for 24 h. Fermented milks were stored at 5±1 °C for 21 days. Samples were tested for bacterial counts and ACE-inhibitory activity of peptide fractions at days 1, 7, 14 and 21 of storage. The results revealed that milk fermentation leads to formation of peptides as the growth factor for LAB and the excess amount of peptides can be accumulated in the medium. In the case of both milks, the lowest IC50 values (the highest hypotensive effect) were observed in the <5 kDa peptide fractions obtained after hydrolysis of milk proteins by Lactobacillus fermentum PTCC 1638. The higher ACE-inhibitory activity of peptide fractions were observed from cultured camel milk peptide fractions than bovine milk counterparts. Furthermore, the lowest IC50 value was obtained 1.073±0.029 mg mL-1 in fermented camel milk after 21 days of storage. Milk fermentation using Lactobacillus fermentum PTCC 1638 is suggested as an economical and practical method to release ACE-inhibitory peptides from milk proteins. Based on our findings, the biological activities of fermented camel milk by Lactobacillus fermentum PTCC 1638 were more pronounced than bovine milk. This showed the potential of fermented camel milk as a novel functional food containing antihypertensive peptides.

Keywords: Fermented milk; Camel milk; Peptides; ACE-inhibitory effect
Title:  
Studying the cellular effects of Mobile phone radiation on HEK293T cells harboring luciferase

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Abstract:  
Wireless technology and mobile phone application are increasing everyday. Studying the potential for external electromagnetic radiation effects on biological responses in cells is now subject of intense research. The cell stress responds can be originated from variety of targets of EMF action on cell, from the cell membrane to the genomic content and signaling systems.

Exposure system consisted of aluminum rectangular waveguide, a signal generator, amplifier and incubator were used to study the effect of exposure (15, 30, 45 and 60 min) on GSH level, caspase3, luciferase, and SOD activities.

The luciferase activity after 30 minute exposure decrease to -21% but when exposure continued to 60 minutes there is a sharp increase in luciferase activity of exposed sample compare to control one (10%). In other hand, the caspase3 activity increase in 15 min exposed plates and reached its maximum activity by exposure.

The increase in luciferase activity after 60 min exposure together with the changes in caspase activity shows that the response mechanisms in HEK293T cell line are activated in presence of applied EMF. The chaperon activity of heat shock proteins might be responsible for alterations in luciferase activity. The GSH level and SOD activity increase in 45 min exposure. Altogether, these results shows that the EMF (electromagnetic field) treatment could have invoke apoptosis in HEK293T cell line meanwhile the induced mechanisms in cell are directed to decrease the shock responses and help the cell to maintain.

Keywords: Mobile Phone, Luciferase, Catalase, caspase3, HEK293T, Cellular effect
Title:
Interaction study of bovine beta-lactoglobulin with a new designed Pd (II) complex by Fluorescence Spectroscopy

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Abstract:
Beta-lactoglobulin (BLG) is one of the main soluble cow’s milk proteins and also a major food allergen, contributing significantly to the overall allergenicity of bovine milk. This protein seems to be resistant and stable to gastric digestion and denaturation.

In the present study we discuss the effect of new designed Pd complex [Pd (bpy) (Et.dtc)]NO3 on the allergenicity and structure of Beta-lactoglobulin (BLG).

We have investigated the interaction of this new synthesized complex with bovine milk carrier protein of BLG using fluorescence at room and physiologic temperatures.

Fluorescence data revealed that the Pd (II) complex is able to quench the intrinsic fluorescence emission of the protein and also may approved that it can bind to the protein and alter the protein structure. The binding parameters of this interaction have calculated using quenching methods at different temperatures.

The results obtained from this study represented that the new synthesized Pd(II) complex can bind to the milk carrier protein of BLG and also can change the tertiary structure of the protein.

Keywords: B-lactoglobulin, Pd complex, fluorescence, quenching
Title:
Study of the binding affinity of anthracycline antibiotic anticancer drug, daunomycin to high mobility group protein B1

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Abstract:
The protein components of chromatin are divided into histone and non-histone proteins. One of the most important families of non-histone proteins are High Mobility Group (HMG) proteins. HMGB proteins are the most abundant group of HMG proteins which participate in various nuclear activities such as replication, transcription and repair. This family consists of four major variants, between them HMGB1 has been studied in detail. Daunomycin is an Anthracycline antibiotic anticancer drug that is most effective on DNA and chromatin. In this work, HMGB1 was purified from rat liver and its binding to daunomycin was investigated using equilibrium dialysis. The plot r versus CF obtained from this study is sigmoid; as CF increases, r rises slowly at first, and then rapidly and finally levels off, indicating that the system reaches a saturation state. The scatchard plot obtained from the study exhibits the positive cooperative binding behavior. Using the equation of $\Delta G^\circ = -RT\ln(K_a)$, the Gibbs free energy change was calculated ($\Delta G^\circ = -6.57$ Kcal/M). The occurrence of a negative Gibbs free energy suggests that the interaction process is exergonic. Association (Ka) and dissociation (Kd) constants were also calculated (Ka: $7.71 \times 10^4$ M$^{-1}$, Kd: $1/3 \times 10^5$ M). In conclusion, the results suggests that daunomycin binds to HMGB1 protein and this binding may affect HMGB1-DNA complex and DNA function in the cell.

Keywords: Chromatin, HMGB1 protein, Daunomycin, Equilibrium dialysis.
Title:
Quercetin Modulates ROS-Induced Oxidative Stress and Notch Signaling Activation in SK-N-MC Cells

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Abstract:
Introduction: There is rising evidence for significance of oxidative damage to the brain in a wide variety of neurodegenerative disorders. It is by now well accepted that reactive oxygen species (ROS)-induced oxidative stress triggers numerous signaling pathways including Notch signaling which are involved in neurodegenerative diseases. Thus, antioxidants aimed at preventing or delaying oxidative stress might be a reasonable choice for treatment of these diseases. Flavonoid quercetin is one of the most prominent dietary antioxidant. In addition to its antioxidant effects, quercetin may be acting by modulating intracellular signaling pathways.

Methods: Cells treated with hydrogen peroxide (H$_2$O$_2$) to induce oxidative stress. The free radical scavenging capabilities of quercetin was studied through MTT assay and antioxidant enzymes activity assay. The extent of lipid peroxidation, protein carbonyl formation and intracellular ROS level as markers of oxidative stress were also studied. Western blot analysis was used to evaluate Notch expression.

Results: Our results showed that pretreatment of the cells with quercetin enhanced the extent of viability in a dose-dependent manner. H$_2$O$_2$ significantly reduced the viability of cells. Moreover, ROS led to reduction of antioxidant enzymes activity. In other words, quercetin protected cells against ROS-induced cell death by down-regulation of lipid peroxidation and protein carbonyl formation as well as restoration of catalase activity. Western blot analysis revealed that ROS activates Notch signaling.

Conclusion: Our results indicated that quercetin can be a promising candidate in antioxidant therapy for ROS-induced neurodegenerative diseases. Collectively, quercetin abrogated the ability of Notch to potentiate oxidative death in neuroblastoma cells.

Keywords: Neurodegenerative disease, Reactive oxygen species (ROS), Antioxidant, oxidative stress
Title: Survivin induces chemoresistance in breast cancer cells

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Abstract:
Introduction: Breast cancer is the second leading cause of death in women after lung cancer. Systemic chemotherapy is the most common modality for breast cancer. Adjuvant therapy has been applied as a desirable strategy to overcome the observed chemoresistance. Survivin as the smallest member of inhibitor of apoptosis protein (IAP) family is a bifunctional protein involved in cell division and caspase inhibition. This protein plays key roles in cancer initiation, tumor progression and chemoresistance to various chemotherapeutics including taxanes. Inhibition of survivin induces apoptosis and thus, sensitizes cancer cells to a number of chemotherapeutic agents. Understanding the role of survivin in chemoresistance has been facilitated by development of survivin inhibitors including deguelin. In the current study, we investigated the role of survivin in chemoresistance to both single and combined treatments of docetaxel with deguelin in MDA-MB-231 breast cancer cells.

Methods: MTT assay was used to measure proliferation of the cells. The amount of apoptosis was assessed using DAPI staining. Survivin expression was investigated in two different levels, gene level by Real-time PCR and protein level by Western blot analysis, both after single and combined treatment.

Results: Our findings showed an IC50 of 10 nM for docetaxel after 48 h incubation with MDA-MB-231 cells. Next, we applied IC50 concentrations of docetaxel along with variable concentrations of deguelin. Combined treatment showed a marked increase in the percentage of apoptosis. Combination therapy also decreased survivin gene expression markedly.

Conclusion: A better understanding of the molecular mechanisms involved in resistance to chemotherapy helps us to find better strategies for cancer therapy. Survivin is an important inducer of resistance to chemotherapy in breast cancer cells which can be considered as a potential molecule for target therapy of cancer. Our results confirm the role of adjuvant therapy in increasing the efficacy of treatment.

Keywords: docetaxel, Deguelin, Survivin, Chemotherapy, MDA-MB-231
Title:
Directional growth of cells in tissue engineering substrate with electric field

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Abstract:
Introduction: Electric and magnetic fields are inevitable environment living organisms. There are also many animal cells through peaceful coexistence with the natural phenomenon because of Electric charge. As low electric field causes the rearrangement or activation of membrane receptors that result some intracellular signals can be changes in the dynamics of the cytoskeleton. In this study, the effect of electric field on the growth and direction cells in the engineered tissue media in order to three-dimensional was performed. Methods: We made an electric field by using a DC power supply with a voltage of 220 volts and a current of 1.0 amperes in a copper coil with different number of rounds throughout a template in the size of cell culture plates. Then the plate containing the biocompatible scaffolds made of PLGA and cells that had been previously prepared, placed in the center of the coil and we put the cells in the incubator at 37 °C and % 5 CO2 for the growth and proliferation of them. Results: In natural state, cultured cells in the media of tissue engineering to move around the media for nutrition and center of media was usually empty of cells, while this condition was interrupted with creating the electric field and forming cells on the scaffold was because of field intensity and the distribution was not uniform. Due to chemical and physical properties inside cells that contain a large number of charge electric, as soon exposure of cells in electric field based on the applied intensity of the electric field, cells formed on the substrate in the direction of the field lines. In this situation the change in pH was not significant. Conclusions: By changing the electric field intensity in different regions of the cell culture plates, cell concentration was different. So that where were higher field strength proliferation and accumulate more, and in the range of low field intensity proliferation and density was low.

Keywords: Bone cells, scaffolds, cell culture, electric field
Title:
Molecular Dynamics Simulation Studies of Antimicrobial Cyclo-RRRWFW and Its Analogs in Water and POPC Bilayers

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Abstract:
Antimicrobial peptides (AMPs) are part of the innate immune system of many organisms. They mostly target bacterial membrane via carpet model and/or formation of pores. Thus, there is a reduced likelihood for bacteria to show resistance towards them. Membrane permeabilization by AMPs depends mainly on both electrostatic accumulation near negatively charged bacterial membrane and hydrophobic insertion into membranes which finally results in a breakdown of barrier function of lipid matrix. To study lipid bilayers-AMPs interactions at atomic levels, molecular dynamic (MD) simulation studies have extensively been used. cyclo-RRRWFW (c-WFW) is an AMP from a group of small hexapeptides which shows good/excellent activity against E. coli. c-WFW interacts strongly with neutral and negatively charged lipid bilayers; however, less information is available on its binding to lipid bilayers in atomistic details. Here, we investigated MD simulations of c-WFW and its analogs, i.e., cyclo-RRRWWF, cyclo-RRRNNalNalRF, cyclo-RRWWRF, cyclo-KKWWKF, and cyclo-RRYYRF in water and POPC bilayer. Simulations were carried out in GROMACS using CHARMM36 force field with lipid, peptides, and TIP3P water model standard parameters in the presence of NaCl. POPC bilayer consisting of around 238 lipids were used and equilibrated for at least 50 ns until the area per lipid converges close to ~ 63–65 Å per headgroup. The peptides are put close to POPC (≤ 10 Å) to reduce the cost of simulations. All simulations were done under P = 1 atm.; T = 310 K. The RMSD and Rgyr values reflecting overall stability of the peptides were computed.

Keywords: Antimicrobial Peptides, Lipid Bilayer, MD simulations, GROMACS
Title: Gene methylation and silencing of SOCS3 in mantle cell lymphoma

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Abstract:

Introduction: The significance of loss of SOCS3, a negative regulator of signaling pathways including those of STAT3 and NF-κB, was examined in mantle cell lymphoma (MCL).

Methods: The protein expression and gene methylation status of SOCS3 were detected using immunohistochemistry/Western blots and methylation-specific PCR, respectively. To evaluate its functional importance, SOCS3 was restored in two SOCS3-negative MCL cell lines using a lentiviral vector.

Results: Loss of SOCS3 protein expression was found in 3/4 MCL cell lines and 18/33 (54.5%) tumors. SOCS3 was found consistently methylated in cell lines (3/4) and tumors (7/7) negative for SOCS3, and was unmethylated in all SOCS3-positive cell line (1/1) and tumors (5/5) examined. Treatment of all 3 SOCS3-negative cell lines with 2′-deoxy-5-azacytidine restored SOCS3 expression. SOCS3 is biologically important in MCL, as lentiviral transfer of SOCS3 in SOCS3-negative cell lines increased their apoptotic activity, downregulated NF-κB-p65, cyclin D1, Bcl-2 and Bcl-xL, and substantially dampened IL-10-induced STAT3 activation. In our cohort (n=33), patients who were ≤ 69 years of age at diagnosis and carried SOCS3-negative tumors showed a trend toward a worse outcome (p=0.1, log-rank).

Conclusion: Loss of SOCS3, a frequent finding in MCL, contributes to the abnormal activation of the STAT3 and NF-κB pathways in these tumors.

Keywords: gene methylation, mantle cell lymphoma, SOCS3, STAT3
Title:
Construction of secretory shuttle vector of p316TDH3

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Abstract:
Introduction: Alpha-factor is a secretory protein in yeast cells. In this study we produce a secretory recombinant plasmid based on signal sequence of alpha-factor for secretion of recombinant proteins in yeast cell. Method: Genomic DNA was extracted from the diploid Saccharomyces cerevisiae yeast. A pair of primer was designed for DNA amplification of the signal sequence of alpha-mating factor. After it, cloning process including PCR product and plasmid digestion was performed and finally recombinant vector was transformed to the competent E.coli cells. Results: Genomic DNA was extracted with 180 µg/ml concentration. Extracted DNA was used as a template for PCR. Two restriction enzyme sites were designed in specific primers, which were Asc1 and Not1. The product with size of 92bp was amplified successfully. The purified PCR product and p316TDH3 plasmid were double digested and then were ligated using T4 DNA-ligase enzyme. The recombinant vector p316TDH3 was transformed to the E. coli DH5α cells and screening of transformed colonies carried out in the presence of an antibiotic marker. This process confirmed by colony-PCR and digestion of extracted plasmid. Conclusions: Using this plasmid as a vector for the expression of desired recombinant proteins, these proteins release in the medium directly without any requirement to use cell lysis methods, this is usually a painstaking work.

Keywords: Alpha-mating factor, Recombinant protein, cloning, secretory signal sequence, PCR
Title:
Rule of the NADPH oxidase pathway in the filamentous fungus Aspergillus fumigatus, a human pathogen

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Abstract:
Reactive oxygen species (ROS) have for a long time been considered as deleterious by-products of aerobic metabolism. However, studies on NADPH oxidases (Nox), a large family of enzymes dedicated to ROS production, have recently shed light on the many important biological roles of ROS. Nox are plasma membrane enzymes that utilize NADPH to generate superoxide (O2-). They have been implicated in defence mechanisms against pathogens in animals and plants, in regulating symbiosis and pathogenicity in fungi, but also in developmental and morphogenetic processes in animals, plants, slime molds and fungi. The presence of at least one or two Nox homologues in all available genome of filamentous fungi oxidase-generated ROS play important roles in fungal physiology and differentiation. Recent studies have shown that ROS are key players of signalling during all stages of fungal development. The exact function(s) of ROS and the role of Nox in fungi are still not completely understood. The role of the Nox enzymes is unknown in human pathogens such as Aspergillus fumigatus, an opportunistic fungus that contains a single Nox enzyme of the Nox1 family. Furthermore, NoxR, the homologue of the gene encoding the regulatory subunit p67phox that activates the Nox genes remains also unstudied. In order to characterize the Nox pathway, we have generated a deletion of the AfNoxA gene. Phenotypic characterization of the invalidated mutants, including asexual and sexual spore production will be presented. To complete this study, the deletion of the AfNoxR, encoding the regulatory protein will be also realized.

Keywords: Aspergillus, NADPH, Human Pathogen, gene
Title: The role of p-glycoprotein in resistance of lung cancer cells to docetaxel and vinblastine

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Abstract:

Introduction: Lung cancer is the leading cause of cancer-related mortality for both men and women. Chemotherapy is now recognized as an important component of treatment for all stages of the disease. However, a major problem in cancer treatment involves the development of drug resistance to a variety of structurally unrelated anticancer drugs, also known as multi-drug resistance (MDR). Among different mechanisms of MDR, overexpression of relevant genes including MDR-1 is a major cause of chemo-resistance. MDR-1 is a gene which encodes a 170-kDa P-glycoprotein, a transmembrane ATP-dependent drug efflux pump. In this study, we investigated the role of p-glycoprotein in chemo-resistance of lung cancer cell line, H1299, by applying Vinblastine in combination with Docetaxel.

Methods: MTT assay was used to measure proliferation of the cells. The rate of apoptosis was assessed using DAPI staining. P-glycoprotein gene expression was investigated in two different levels, gene level by Real-time PCR and protein level by Western blot analysis both after single and combination therapy.

Results: Incubation of non-small cell lung carcinoma, H1299 cells, with docetaxel showed an IC50 of 30 nM after 48 h. Applying docetaxel in combination with vinblastine decreased the viability of the cells from 50% to 23%. Combination therapy of vinblastine and docetaxel also showed a marked decrease in MDR-1 gene expression (p < 0.05).

Discussion: Our results implicated that MDR-1 is an important gene in induction of chemo-resistance in lung cancer cells. Our findings revealed a significant increase in efficacy of our chemotherapeutic agents by decreasing the MDR-1 gene and protein expression. Hence, P-glycoprotein can be noticed as a novel target for eradication of cancer cells.

Keywords: Chemotherapy, Multi-drug resistance (MDR), H1299 cell line
Title: Theoretical study of the functionalized carbon nanostructures as nanovectors for drug delivery

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Abstract:

Introduction: Carbon nanotubes (CNTs) are nano-substances that have raised great prospects in a number of different applications. Functionalised CNTs (f-CNTs) are new systems for a variety of potential biomedical applications such as vaccine, drug and gene delivery systems. These systems are highly soluble in water, that allows the formation of complexes with biological substrates. Therefore, the covalently f-CNTs are promising systems for attaching biomolecules aiming nanobio-related applications.

Method: Theoretical investigations were performed to study the structures and properties of carbon nanoclusters namely, fullerene (C60) and CNTs. Ground state geometries were optimized at the DFT level using the B3LYP/6-31G(d) functional basis set. Theoretical calculations were carried out to study the effects of covalently binding of antitubercular compounds of ethionamide and pyrazinamide to functionalized fullerenes (f-C60) and f-CNTs. The binding energies, energies of salvation, the charge transfers between the functional groups and carbon nanoclusters and quantum-chemical molecular descriptors were computed.

Results: Data indicate that it is feasible to covalently bind both ethionamide and pyrazinamide to f-C60 and f-CNTs. The results from binding energies show the stronger and more favorable binding of both ethionamide and pyrazinamide to f-C60 than to f-CNTs. Also, the affinity of ethionamide for both of f-C60 and f-CNTs are comparable with pyrazinamide. Based on solvation energies data, both of f-CNTs and f-C60 are capable to solvate in water but the solubility of f-CNTs is higher than f-C60.

Conclusion: Data imply the thermodynamic stability towards covalent attachment of antitubercular drug molecules onto f-CNTs and f-C60. Our findings suggest that the incorporation of carbon nanoclusters-based antitubercular drugs provide a route for the potentially intracellular delivery of drugs as nanovectors.

Keywords: nanovectors, CNTs, fullerene, pyrazinamide, ethionamide
Title:
The Effects of the chaperone property of argentine (small molecule) and chemical chaperones glycerol and α-Crystallin in preventing aggregation of alpha-lactalbumin in the crowded system

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Abstract:
Aggregation is phenomenon in which protein loses its native structure and adopts an abnormal conformation. Misfolded protein aggregates by binding to other proteins with similar situations which causes amyloidosis diseases such as Alzheimer depending on its location. Molecular chaperone are classified in a group of cellular proteins which mediate correct folding of polypeptides. They bind to exposed hydrophobic surfaces of unfolded proteins, avoid unsuitable interactions which may lead to aggregation. Glycerol belongs to the polyols family of chemical chaperones, which are known to stabilize protein conformation and prevent aggregation. α-Crystallin is a member of the small heat shock proteins family that has been shown prevents aggregation. Arginine is small molecule that has been proven to act as molecular chaperon. Physiological media in living cells are crowded due to the high total concentration of macromolecules such as carbohydrates. In this study, comparison of the chaperoning action of α-Crystallin and glycerol and arginine in preventing aggregation of α-lactalbumin in crowded system examined using ANS binding, intrinsic fluorescence, CD spectroscopy and Electrophores. Glycerol and arginine showed significant effect in preventing protein aggregation. Arg had positive effect to the chaperone action of α-crystallin in the presence of dextran, the effect of Arg on the chaperone ability of α-crystallin was less and the chaperone ability of glycerol and arginine decreased. Dextran induced the aggregation of protein and structural change chaperone. However the result demonstrated the higher activity of Arg in the presence of dextran. This is likely due to the better stabilizing effect of Arg on protein structure and environment. the effect of Arg on the activity of the α-crystalline in crowded system, it can provide mechanism to protect cells against aggregation.

Keywords: chaperone _ aggregation _ Molecular crowding _ a-lactalbumin _ α-Crystallin
Title:
Dispersive liquid-liquid microextraction and spectrofluorimetric determination of aluminum in fish

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Abstract:
Background: Aluminum(Al) is a non-essential and toxic metal which human can be frequently exposed to by consuming Al containing medicines, inhalation of atmospheric dust, food, drinks, etc. There are some events showing that, Al can be involved as a causative factor in some diseases like Alzheimer's disease, Parkinson's disease, cancer and diabetes. Therefore, there is a great demand to introduce simple and accurate analytical methods for determination of trace amounts of Al in different samples specially foodstuffs. In this work a dispersive liquid-liquid microextraction technique has been developed and optimized for spectrofluorimetric determination of Al in fish.

Methods: Approximately 1 g of homogenized fish sample was digested by adding 10 ml of nitric acid 50%. After 30 minutes, 100 µl of digested sample was transferred into another tube and mixed with 10 ml distilled water, 1 ml of 8-hydroxyquinoline 1 \times 10^{-4} M and 1 ml of sodium chloride 4%(w/v). After that, pH of the mixture was adjusted to 7.00 by adding diluted hydrochloric acid. Then the mixed solution of 600 µl of acetonitrile (dispersive solvent) and 150 µl of chloroform (extracting solvent) was quickly injected into the tube and a cloudy mixture was obtained. After centrifugation of the obtained mixture, the collected organic phase at the bottom of the tube was analyzed by a spectrofluorimeter at excitation/emission wavelengths of 400/550 nm. Results: All variables affecting the extraction step, such as extracting and dispersive solvents, pH, time of complex formation, sample volume and speed of centrifugation were optimized. Then under the optimum conditions, the method was validated in terms of linearity, LOD, LOQ, accuracy and precision, according to the ICH guideline. Obtained results indicated that, complex formation was completed after 30 minutes and the optimized mixture of extracting and dispersive solvents was consisted of 150 ml chloroform and 600 ml acetonitrile, respectively. Under the optimum conditions, the method was linear in the concentration range of 20 – 500 ng/ml with correlation coefficient of more than 0.999.

Conclusion: In present study a DLLME technique has been successfully applied for the extraction of trace amounts of Al from fish samples as a prior step to determination of Al by spectrofluorimetry.

Keywords: dispersive liquid-liquid microextraction, Spectrofluorimetry, Aluminum, Fish samples
Title:
Betaine acts as an antioxidant and methyl donor agent versus levodopa-induced oxidative stress and hyperhomocysteinemia in the rat kidney

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Abstract:
Betaine has recently been shown to bear antioxidant and methyl donor effects in our previous reports. In the present study, we examined methyl donor and antioxidant properties of betaine in a Parkinson’s disease (PD) model in rats. Sprague–Dawley male rats were divided into levodopa (LD), Betaine (Bet.), levodopa plus betaine (LD/Bet.), levodopaplus benzerazide (LD/Ben.), levodopa plus betaine-benserazide (LD/Bet.-Ben.) and control groups. The experimental groups received LD 300 mg/kg, Bet. 1.5% w/w of the total diet, Ben. 75 mg/kg, and vehicle to controls for 10 consecutive days via gavage. Plasma total homocysteine concentration decreased significantly in betaine-treated rats when compared to LD- and benserazide-treated groups. TBARS concentration (as a lipid peroxidation marker) reduced statistically in betaine group in comparison with LD and LD/Ben. groups. Catalase activity increased significantly (in order compensatory) to suppress oxidative stress in LD-treated rats when compared to controls. Superoxide dismutase activity significantly decreased in LD-treated group when compared to LD/Ben. group. However, there was not any significant difference in glutathione peroxidase activity among the groups. These findings indicate that LD and LD/Ben. (as acent PD treatment drug) beside the toxic effects in brain due to hyperhomocysteinemia and oxidative stress (our unpublished data), induce oxidative stress in the rat kidney, as well. In contrast, betaine acts as an promising antioxidant and methyl donor agent versus LD-induced complications. Therefore, this research highlights the therapeutic antioxidant and methyl donor effects of betaine in line with our previous reports in cerebellum, testis, ovary, liver, and brain of rats.

Keywords: Parkinson’s, Betaine, Levodopa, Benserazide, Homocysteine, Kidney.
Title: Recombinant expression and refolding of anti-EGFR Single domain antibody

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Abstract: Introduction: Antibodies have turned out to be important tools in biomedical research and have been found numerous applications in the detection and targeting of variety of molecules with high affinity and specificity. Protein aggregation is a major challenge encountered in production of recombinant proteins including recombinant antibodies. Hence, various protocols have been proposed to solubilize inclusion bodies. The aim of this study was to investigate the solubilization, refolding capacity and binding affinity of engineered anti-EGFR single domain antibody (sdAb). Material and method: Variable region of heavy-chain of c225 anti-EGFR antibody was expressed in E. coli. After cells were sonicated, the pellet was solubilized with 8M urea in 10mM Tris–HCl (pH=8.0) overnight at 4°C. The supernatant was then applied to a Ni-NTA column for purification and bound proteins were eluted by increasing concentrations of imidazole. Refolding was carried out by dialysis against refolding buffer containing 4, 2 and 1M urea and different concentration of L-arginine, GSSG and GSSG. After refolding, the soluble and insoluble fractions were separated by centrifugation and protein concentration was determined by Bradford’s assay. Finally the binding activity of refolded sdAb was detected by ELISA. Results and discussion: PCR amplification of heavy chain variable domain resulted in a 380bp product. Expression of cloned fragment in E. coli Bl21 leads to production of a 12.2kD protein as shown by SDS-PAGE analysis of IPTG-induced bacteria. Refolding experiments revealed that the highest yield of active protein was obtained in 0.5mM GssG, 5mM GssH and 300mM l-arginine. ELISA analysis showed that recombinant sdAb can bind to EGFR on A431 cells with high affinity. Conclusion: SdAb could be prepared using E. coli and further in-vitro refolding.

Keywords: Single-domain antibody, EGFR, In-vitro refolding
Title: Comparing Energy Barrier of Glycation and Fructation

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Abstract: Fructose is commonly used as an industrial sweetener and has been excessively consumed in human diets in the last decades which cause the development of metabolic disorders. Fructose compared to glucose has been found to be a more potent initiator of the glycation reaction. In protein glycation a reversible Schiff base formation is followed by Amadori rearrangements and generation of various glycated end products (AGEs). Therefore, the activation energy for both glucose and fructose has been achieved in this investigation. Bovine serum albumin supplemented with 500 mM of glucose and fructose was incubated for 10 weeks in different temperature (4, 15, 25, 37 and 40°C). The protein glycation process was studied by both intrinsic and extrinsic fluorescence strategies using Carry Eclipse spectrofluorometer. Fluorescence studies of BSA samples glycated for over 10-weeks of incubation along with non-glycated BSA samples as control, revealed an increase in the fluorescence intensity for both glucose and fructose. Subsequently the activation energy of a reaction was determined from the slope of Arrhenius plot which has been obtained by plotting the logarithm of the rate constant, K, versus the inverse temperature, 1/T. The results are discussed from the point of view that fructose (86.329 kJ/mol) rather than glucose (135.201 kJ/mol) is more extensively involved in glycation.

Keywords: Glycation, Fructose, Glucose, bovine serum albumin.
Title:
Use of immobilized Bacillus subtilis natto cells in calcium alginate matrices on MK-7 production

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Abstract: INTRODUCTION Menaquinone-7 (MK-7) a category of vitamin K has shown a significant effect on preventing cardiovascular and osteoporosis which are the major health issues. The rate of suffers from these diseases is rising rapidly, urging the need for production of highly concentrated MK-7 supplements. MK-7 can be produced mainly by the liquid fermentation of Bacillus subtilis natto. Cell immobilization technique due to its high stability against contamination and high productivity attracted attention for production of many bio-products. In this study, therefore, the possibility of immobilization of Bacillus subtilis natto cells for MK-7 production was investigated and the result was compared to un-immobilized culture condition. METHOD Fermentation was carried out at 40 °C for a period of 3 days in 25 mL round bottles. fermentation media was consisted of 5% (w/v) yeast extract, 18.9% (w/v) soy peptone; 5% (w/v) glycerol and 0.06% (w/v) K$_2$HPO$_4$. Cell immobilization was performed by using sodium alginate. A 2% (w/v) of the sterilized sodium alginate solution were thoroughly mixed with the Bacillus subtilis natto cells. Beads were prepared by droplet from a pipette about 5 mm diameter in a sterilized 6% (w/v) calcium chloride solution. A mixture of 2-propanol and n-hexane (2:1, v/v) was used to extract MK-7 from each. High performance liquid chromatography (HP 1050, USA) equipped with a UV detector and C$_{18}$ Gemini column at 40°C (5 µm, 250 × 4.6 mm, Phenomenex, USA) was used for measuring MK-7 concentration. RESULTS The amount of MK-7 increased gradually in both immobilized and un-immobilized systems during the fermentation period. The results are shown in Figure 1 and the data indicated that MK-7 production reached a maximum level after six days of fermentation. It was observed that the MK-7 production with immobilized cells in calcium alginate was less than the un-immobilized cells. In un-immobilized cell condition, MK-7 concentration obtained was 56 mg/L after 6 days, while in the immobilized system 48 mg/L MK-7 was produced at the end of the fermentation period. There was a slight difference in pH profile of immobilized and un-immobilized cells (Figure 2). This difference in pH profiles can be due to different metabolic activities of cells within the calcium alginate matrix and un-immobilized condition. The cell leakage from the matrix was gradually increased with increase of fermentation time. Increase in cell leakage can be related to cell growth within the beads which is reported as one of the major challenges in cell immobilization technology. However, the beads were not disintegrated during the operation batch. CONCLUSION Based on the achieved results, alginate-entrapped cells showed the ability to produce considerable amounts of MK-7. This finding eases of conversion of batch fermentation on continuous mode without cell washout. Slightly lower MK-7 level during the fermentation period in immobilized system can be due to the small pore size of the beads which inhibited the substrate diffusion toward the cells and hindered the release of MK-7 during the batch fermentation. For the embedded cells, the substrates have to migrate through the matrix between the cells in order to maintain the optimum growth and MK-7 formation. Therefore, further investigation need to be carryout on intraparticle diffusion on the activity of immobilized Bacillus subtilis natto cells for MK-7 production.

Keywords: MK-7 production, calcium alginate matrices, Bacillus subtilis natto
Title: Design and Molecular modeling of a humanaized scFv antibody against hTNF-α and investigation of their interactions

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Abstract: TNF-α is a multifunctional cytokine mainly secreted by stimulated macrophages to activate controlling systems involved in cell proliferation, differentiation, inflammation, death and immune regulation. Although normal serum level of TNF-α is very important to regulate immune responses, its elevated level has been implicated in the pathogenesis of chronic inflammatory, autoimmune, and infectious diseases. Therefore, targeting TNF-α is an effective therapeutic strategy in the control and treatment of such diseases using corresponding antibodies. Most of the marketed anti–TNF-α antibodies have side effects making the treatment process difficult. The aim of the current work was to design and model a novel humanaized scFv antibody against hTNF-α and investigate their interactions.

Methods: Sequence of murine single chain antibody (D2) with the highest binding affinity toward TNF-α was humanized virtually and named hD2-scFv. 3D model was generated using SWISS-MODEL server based on the suitable template. The model was subjected to molecular dynamics simulation using AMBER package and the model with lowest energy was extracted for docking procedure. The TNF-a was docked onto the binding site of model structure of hD2-scFv. The TNF-a was docked onto the binding site of model structure of hD2-scFv using ZDOCK program and the interactions in the complex of ligand–antibody were identified.

Results: The result of docking study was analyzed using programs like LigPlot and the important interactions were identified. Conclusions: The result of this study can be used in identifying pharmacophore responsible for molecular interaction and can guide further experimental studies for designing novel anti TNF-a antibodies with improved affinities.

Keywords: tumor necrosis factor-alpha, Molecular modeling, scFv
Title: Functionalized cationic silica Nanoparticles as biocompatible carriers by Stimuli- responsive Nanovalves as double anticancer drug delivery systems

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Abstract: In this paper a novel temperature and pH-sensitive cationic mesoporous silica nanoparticles (MSN) composed of poly (N-isopropylacrylamide-methacrilic acid -N,N-dimethylaminoethylmethacrylate)(NIPMADM-MSNs) was fabricated through the facile in situ polymerization method. First, a positively charged ionic liquid monomer was prepared from N,N-dimethylaminoethylmethacrylate (DMAEMA) and 3-chloropropyl trimethoxysilane as coupling agent under mild conditions. Then methacrylic-type ionic liquid terpolymer was synthesized by free-radical copolymerization of MAA, NIPAAM and polymerizable ionic liquid. The structure of the novel ionic liquid terpolymer was characterized and confirmed by FT-IR and 1H-NMR spectroscopy. Finally stimuli responsive organic-inorganic nanocomposite was obtained by reaction of the obtained IL with SiO2. The prepared nanoparticles were characterized using SEM, Zetha potential and TGA methods. SEM results reveal the uniformity in size/shape of silica particles with a mean diameter of approximately 60nm. The prepared nanoparticles were used as double delivery system for two model anticancer drugs, Doxorubicin (DOX) and Methotroxate (MTX). The both drugs could be loaded effectively to functionalized MSNs channels through electrostatic interactions between drug and matrix to generate a stimulus responsive controlled release system. The thermo and pH-driven “gate-like” effect was studied by simultaneous in vitro release of both entrapped drugs at different temperature and pH value. A novel HPLC-UV method was applied for simultaneous determination of two anticancer drugs DOX and MTX. Complexation of DOX and MTX with modified MSNs yielded a drug delivery system affording a pH-triggered release of entrapped drugs at weak acidic conditions. Also the drug release at 41 °C is faster than 37 °C. The results indicated that at physiological conditions (pH 7.4), because of the formation of ionic interaction between carrier and drugs negligible release has been observed. While the protonation of carboxyl groups at mildly acidic condition resulted in a faster dissociation of copolymer-DOX and MTX complex, leading to an accelerated release of both drugs at mildly acidic conditions (pH 4). Therefore co-delivery of two anticancer drugs with distinct well-controlled release profiles was achieved from this double delivery system. A549 and MCF7 cell lines were incubated with the blank and polymer-drug complexes at various concentrations for 72 h .In vitro cytotoxicity assay showed that the blank carrier were highly biocompatible and was suitable as drug carriers. DOX@MTX@NIPMADM-MSNs exhibited higher antitumor activity after 72 h culture in comparison with MTX@DOX in free form.

Keywords: Gate keepers, Stimuli-responsive, Double Delivery System, Anticancer, Cationic Silica Nanoparticles
Title:
Engineering of the C-terminal domain of Pseudomonas aeruginosa elastase: engineering of the second and third calcium-binding sites

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Abstract:
Neutral proteases (NPs) are zinc metalloproteases with different industrial applications, but autolysis at high temperatures is a limitation in this regard. Elastase is drastically active in organic solvents, and thermolysin is a thermostable enzyme. To obtain an enzyme mutually present both thermal and organic solvent stabilities, an extended surface loop in elastase, which is coordinate to one calcium, was replaced with the corresponding region in thermolysin, comprising three calcium binding sites. The enzymes were expressed in E.coli, and purified by ion exchange chromatography, then their stabilities against temperature, denaturants, metal ions, acidic pHs, and NaCl were investigated in comparison to the wild type elastase (WT). Accordingly, measurements of half-life of inactivation in the presence and absence of 20 mM CaCl2 at different temperatures (65-90 °C) revealed a remarkable stabilization of the chimeric enzyme so that T50, the temperature for which a 30 min incubation reduces the enzyme activity by half was improved as 25 °C. To further assess the thermostability, thermodynamic parameters of the inactivation process including activation energy (Ea), ΔH#, ΔG# and ΔS# were calculated. Calculation of thermodynamic parameters of inactivation indicated that improved resistance of chimeric enzyme in due to increased ΔH#, implying the Ca-dependent increase of the number or strength of enthalpy-driven interactions that have to be broken during activation. Moreover, stability of the chimeric enzyme in the presence of metal ions and NaCl, in addition to against acidic pH, denaturants was notably improved in comparison with the WT enzyme.

Keywords: Elastase, Thermolysin, thermal stability, metal ions, denaturants, NaCl.
Title:
Energy oscillation in apoptosis and differentiation processes

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Abstract:
Apoptosis and differentiation are physiological processes that share many common features, for instance, chromatin condensation, cytochrome c release and caspase activation. Recently several studies have shown that differentiation process pass through a death centric pathway as apoptosis process. Apoptosis has been known as an ATP-dependent process and also differentiation is associated with energy changes. In this study we address to the role of energy oscillation in these processes.

Mouse Embryonic Stem Cells (mESCs) were directly differentiated to cardiac cells in the presence of ascorbic acid. In parallel with differentiation, apoptosis were induced by doxorubicine as an apoptogenic compound. Sampling was performed in timeseries of 6h, 12h, 24h, and 48h. Changes in energy were examined by measuring cellular ATP level using a luminescent system and complex I activity by an enzymatic method. Simultaneously, activation of caspases was assayed using a bioluminescent kit at the time series as mentioned above.

Our study reveals that energy oscillation has a critical role in apoptosis and differentiation processes. Energy increases in apoptosis and differentiation processes but in a different manner. In both processes we observed caspase activation when the cells generated most of cellular ATP. Therefore we conclude caspases behave in an ATP-dependent manner in apoptosis and differentiation processes.

Keywords: Stem cell, Cardiac differentiation, Apoptosis
Title:
Apple peel derived ursolic acid: anti aging agent with restriction caloric effect

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Abstract:

BACKGROUND:
Ursolic acid (UA) is a lipophilic pentacyclic triterpenoid that contributes to the waxy coats on apples, other fruits, and many herbs, including some folkloric herbal medicines for diabetes. It has been recently identified ursolic acid during a screen for small molecule inhibitors of skeletal muscle atrophy. Skeletal muscle atrophy is characteristic of starvation and a common consequence of aging. There is idea that aging may be linked to energy expenditure and it is intuitively attractive research field.

Material & Methods

Ursolic acid (200 mg/kg) or vehicle alone (corn oil) via I.P administrated to C57BL/6 mice and skeletal muscle tissue’s isolated for measuring ATP/ADP ratio using firefly luciferase assay and then both the test and control samples analyzed with ANOVA test and compared with each others.

Results

ATP/ADP level in 2 group (control and test) are measured and analyzed with ANOVA test that shows a significant difference between the groups (P-value < 0.001).

Conclusion

In the view of the prominent role of Caloric Restriction (CR) in prolonging the longevity of organism from yeast to mammals, and also in lower blood glucose levels, decline in glycogen and fat stores, enhanced responsiveness to insulin, lowered body temperature and diminished reproductive capacity, increases mitochondrial biogenesis in liver, fat and muscles. here, we tried to determine whether UA can be considered as a possible anti-aging marker that may change the ATP, ADP and ATP/ADP ratio or not. For this, skeletal muscle tissues were lysed and energy charge content was measured using firefly luciferase assay. Therefore, it is suggested that UA acts as a caloric restriction marker which can accelerate catabolism/anabolism.

Keywords: ursolic acid, anti-aging, caloric restriction, ATP/ADP
Title:
Design and Biosynthesis of Genetically Engineered Biomimetic Peptides as Gene Transfer Vector

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Abstract:
Gene therapy is defined as the treatment of human disease by the transfer of genetic material into specific cells of the patient. The main obstacle of successful gene transfer into eukaryotic cells is the lack of a suitable carrier. The main features of a suitable vector for clinical application are low cytotoxicity and immunogenicity, high efficiency, tissue specificity and cost effectiveness. Many studies about gene carriers are ongoing and significant progress has been achieved. One of the most important carriers used in gene transfer into cells are synthesized peptides by genetic engineering techniques. These peptides are composed of several domains which are inspired by peptide sequences found in nature, especially viruses.

In the present study we have designed and prepared a new peptide using recombinant technology with the purpose of overcoming the cell barriers. Design of peptides was done by I-TASSER modeling server. Two repeats of 16mer peptide of histone H1 was used as a DNA binding domain. The peptide was also included a SV40 large T antigen NLS which is prospecting to help the cargo to reach the nucleus and included HIV 41 glycoprotein as fusion part. The His tagged peptides were prepared in BL21 (DE3) pLysS and purified using Ni-NTA resin. Peptide high expression was proved by SDS-PAGE and western blotting. The formation of peptide complex with a reporter plasmid (luciferase) was examined by retardation assay. The results demonstrated that the vector is able to condense plasmid DNA into nanosize particles.

Keywords: nonviral gene delivery, recombinant technology, Biomimetic Vector
Title:
Cloning, expression, and purification of DOF 4.2 zinc finger domain

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Abstract:
Background and Aims: DOF (DNA-binding with one finger) DNA-binding transcription factor family is a member of C2C2 zinc finger proteins unique to plants. They are responsible for different plant specific phenomena like germination, dormancy, light and defense responses. Purification of DOF domain proteins for solving their structures still remains a big challenge as this proteins bind tightly to their putative DNA sequences. Our aim is to clone the zinc finger domain of DOF 4.2 for the purpose of large scale protein production and purification, which can then be used in biophysical studies to identify key interactions involved in interaction with partner DNA. Methods: Arabidopsis thaliana was used for total RNA extraction followed by synthesis of cDNA library using reverse transcriptase PCR reaction. The gene of interest was amplified by PCR and cloned in pGEX expression vector. The construct was transformed into E. coli BL21 for high level expression of the recombinant GST-ZF DOF4.2 fusion protein. SDS-PAGE was used to detect the expression of the protein before purification steps by affinity and size exclusion chromatography. Results: DOF4.2 zinc finger domain coding region was cloned into the glutathione S-transferase (GST) containing vector named pGEX-6p-1 using the cDNA library prepared from plant Arabidopsis thaliana. Surprisingly, it was found by sequencing that there are some mutations in wild type of plant (G125A and S145A). The mutations were analyzed and the construct was transformed into E.coli BL21 for high level expression of the GST-ZF DOF 4.2 fusion protein. Conclusion: The results of the current investigation showed that it is possible to successfully clone and express for the first time the naturally mutated zinc finger domain of DOF 4.2 plant transcription factor as a GST fusion protein. The expressed fusion protein was mainly isolated from the soluble fraction prepared from the cell lysate of transformed E. coli BL21 cells. Affinity chromatography, followed by size exclusion chromatography was applied to prepare high purity DOF4.2 ZF domain.

Keywords: Transcription factor, DOF proteins, Zinc finger domain, plasmid, cloning
Title:
Cloning, expression, and purification of DOF2.1 zinc finger domain

Authors:
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Abstract:
Background and Aims: DOF DNA-binding transcription factor family is a member of zinc finger (ZF) containing proteins unique to plants. They are associated with different plant specific phenomena like germination, dormancy, light and defense responses. Until now there is no report based on determination of three dimensional structure of this group of protein. Our aim is to clone the zinc finger domain of DOF 2.1 for the purpose of large scale protein production and purification, which can then be used in biophysical studies to elucidate their mode of interaction with DNA. Methods: The whole body of the plant Arabidopsis thaliana was used for total RNA extraction, which in turn used in reverse transcriptase PCR reaction to prepare cDNA library. The cDNA of interest was amplified by PCR and cloned in pGEX expression vector. The generated construct was transformed into E. coli BL21 for high level expression of the recombinant GST-ZF DOF2.1 fusion protein. SDS-PAGE was used to detect the expression of the protein before purification steps by affinity and size exclusion chromatography. Results: DOF2.1 zinc finger domain coding region was cloned into the gluthatione S-transferase (GST) containing vector named pGEX-6p-1 using the cDNA library prepared from plant Arabidopsis thaliana. Following the confirmation of the construct by sequencing, the plasmid was transformed into E.coli BL21 for high level expression of the GST-ZF DOF 2.1 fusion protein. Conclusion: The results of the current investigation showed that it is possible to successfully clone and express the zinc finger domain of DOF 2.1 plant transcription factor as a GST fusion protein. The expressed fusion protein was mainly isolated from the soluble fraction prepared from the cell lysate of transformed E. coli BL21 cells. Affinity chromatography, followed by size exclusion chromatography was applied to prepare high purity DOF2.1 ZF domain.

Keywords: Transcription factor, DOF proteins, Zinc finger domain, plasmid, cloning
Title:
Effect of glycine on hemoglobin glycation by glucose, glucose 6-phosphate or fructose

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Abstract:
Aim/Objectives. Glycation is a nonenzymatic process that happens when reducing carbohydrates like glucose, fructose, and glucose 6-phosphate reacts with biomolecules like proteins, lipids, and nucleic acids. Protein glycation has an important role in diabetic complications such as nephropathy, retinopathy, cataract, atherosclerosis, neurodegenerative disease, and etc.; therefore lowering the rate of protein glycation could help diabetic patients. Methods. We investigated hemoglobin (Hb) glycation by glucose (Glc), glucose-6-phosphate (G6P) and fructose (Fru) in the presence of glycine (Gly). Hb was incubated in phosphate buffer saline for four months at 37 °C with or without Glc, G6P or Fru in the presence or absence of Gly. Samples were gathered every two weeks and maintained at -80 °C. After 4 months samples were investigated by fluorometry, circular dichroism (CD) and electrophoresis. The fluorescence intensity of advanced glycation end products (AGEs) using fluorometry, secondary structure of protein using a CD and electrophoretic mobility of samples using electrophoresis was followed. Results showed that Hb structure changed due to the glycation. AGE formation was increased and the electrophoretic mobility of the Glycated Hb was more than the native protein. All the named changes were reversed in the presence of Gly; however the effect of the three named sugar was different in the glycation of Hb and the effect of Gly on prevention of glaciation by each of them was also different. Conclusion. In the present research comparison between the effect of each sugar on Hb glycation and the antiglycating effect of Gly is presented.

Keywords: Effect of glycine on hemoglobin glycation by glucose, glucose 6-phosphate or fructose
Title:
The effect of some anti-glycating agents on the early, intermediates and advanced glycated end products of albumin

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Abstract:
Aim/Objectives. Glycated albumin (g-Alb) is one of the most important Amadori products. Elevated levels of g-Alb, which is determined by the fructose amine test, cause permanent damage connected with the metabolic disorders observed in diabetes mellitus, such as retinopathy, nephropathy, neuropathy and coronary artery disease. In this study, we compared the effect of crocetin (Crt), glycine (Gly) and N-acetyl cysteine (NAC) on the glycation of albumin and formation of glyoxal (GO), methylglyoxal (MG), pentosidine and advanced glycation end product (AGEs).

Methods. Albumin was extracted from rat serum using TCA and ethanol, using the method of Ohkawara et al.; then it was incubated with glucose and each of the named ligands. After three weeks, GO and MG were determined by HPLC; and after three month the AGEs and pentosidine were determined by fluorometry and HPLC, respectively.

Results. Formation of the mentioned components was shown in the presence of Glc alone; however, Crt, Gly and NAC inhibited the formation of these products with various degrees. Conclusion. Various inhibitory effects of the named ligands on the Alb glycation and the formation of glycated products at different stages were observed. Thus the mechanism of their inhibitory effect is different.

Keywords: The effect of some anti-glycating agents on the early, intermediates and advanced glycated end products of albumin
Title: Methionine Synthase Gene polymorphism (MTR): Association with Plasma

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Abstract: Retinoblastoma is the most prevalent intraocular solid tumor in the baby under the age of six. Mechanism of carcinogenesis by Subnormal DNA methylation is elaboration. When baby exposed to low measure of maternal folate Pending retinogenesis may have enhanced uracil misincorporation, hypomethylation and, as a affect, be more probably to extension postzygotic mutations in RB1 disease. DNA methylation is Climacteric for regulating gene expression and gene Accuracy. The profusion of MTR A2756G (rs1805087) polymorphism was analogy between retinoblastoma patients and persons without history of neoplasias.

Material and Method: A total of 705 persons were inclusived in the experiment. The polymerase chain reaction restriction fragment length polymorphism technique was used to genotype the polymorphism. For statistical analysis, the chi-square test (univariate analysis) was used. Plasma levels of folate and vitamin B12 were determined using a radioassay kit (Ciba-Corning, Walpole, MA).

Result: Using univariate analysis, the results did not show significant differences in allelic or genotypic distributions. Multivariable analysis showed that tobacco and alcohol consumption (P< 0.05), AG genotype (P = 0.017) and G allele (P = 0.026) may be predictors of the disease and a higher measure of the G polymorphic allele was apperarented in men with retinoblastoma compared to male controls (P = 0.006).

In conclusion: This experiment demonstrated that MTR A2756G polymorphism can not only affect homocysteine concentration, but also can adjustment therapeutic replies to diverse dosages of FA supplementation. our data provide voucher that supports an association between the polymorphism and the risk of retinoblastoma.

Keywords: Retinoblastoma; Polymorphism; Folate metabolism; MTR gene
Title:
Correlation of Three-dimensional Structure with the Antibacterial Activity of Aurein 1.2

Authors:
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Abstract:
Introduction: Recent interest in the search for alternative therapeutics is growing because of the drug resistance problem with traditional antibiotics. Antimicrobial peptides have attracted much attention because of their favorable properties, such as rapid killing, wide spectrum and rare development of drug resistance. Aurein 1.2 is one of these amphipathic peptides that has 13 residues with antibacterial and anticancer activity. Molecular dynamics simulations can provide valuable information about the various stages of AMP/lipid interactions. Short-timescale simulations have elucidated phenomena that are dominant when the peptide is in a surface-binding state, a trans-membrane state, or when multiple peptides are assembled into a pore.

Method: Actually we are performing molecular dynamics simulations of the interactions of helical anti-microbial peptides, aurein 1.2 and its analogue, with dipalmitoylphosphatidylcholine (DPPC) lipid bilayers by gromacs software package. In this article, by studying the structure of this peptide and one of its nontoxic analogues inside the bilayer, we investigate the correlation of 3-D structure with the antibacterial activity of the peptide.

Results: During our simulations, although both peptides form $\alpha$-helix, there is structural difference between the nontoxic anchor and the toxic antimicrobial peptide.

Conclusions: From the simulation of anti-microbial peptide aurein 1.2 with DPPC bilayers, we will expect key factors that dominate the peptide/lipid interaction. Comparing the structure and flexibility of homologues nontoxic and antibacterial peptide guide us to design new antibacterial peptide.

Keywords: Antimicrobial Peptide, Membrane, Molecular Dynamics Simulation.
Title:
Design, synthesis and toxicity assessment of novel 4'-(4-(methylsulfonyl) phenyl)-3' p-substituted phenyl -4'H-spiro [indene-2, 5'-isoxazol]-1(3H)-one as selective COX-2 inhibitors and study of their COX-2 binding modes using docking studies

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Abstract:
Introduction: Selective COX-2 inhibitors, such as celecoxib have been developed as a new generation of NSAIDs with reduced GI side effects. However, rofecoxib and valdecoxib were withdrawn from the market because of an increased risk of cardiovascular problems. Recent studies specify the place of COX-2 inhibitors in cancer chemotherapy. Moreover, COX-2 is overexpressed in many solid tumours and inhibition of this enzyme in vitro using specific COX-2 inhibitors has shown that COX-2 is a potential target for novel cancer therapies.

Method: Docking studies were performed by GOLD software. The synthesis of designed compounds was started from p-substituted-benzaldehydes that converted to p-substituted-benzaldoximes, and then (E)-2-(4-(methylsulfonyl)- benzylidene)-2,3-dihydro-1H-inden-1-one was obtained by reaction of 2,3-dihydro-1H-inden-1-one with 4- methylsulfonyl benzaldehyde. The final reaction was 1, 3-Dipolar cycloaddition reaction of p-substituted-benzaldoximes with mentioned chalcone in biphasic medium of aqueous sodium hypochlorite and chloroform. Toxicity evaluation of synthesis compounds against MCF-7, T47D, HT29 and HFF cell lines was evaluated using the MTT assay.

Results: Molecular modeling revealed that the designed compounds were well incorporated in the active pocket of COX-2 enzyme. As a result a new series of indenone spiroisoxazolines compounds was synthesized as selective cyclooxygenase-2 inhibitors. The purity of synthesized compounds was tested by chromatography. The structures of synthesized compounds were confirmed by ¹HNMR, IR and MS spectrometry. The MTT assay of the synthesized compoundexhibited remarkable antiproliferative effects comparable to the positive controls.

Conclusions: Docking simulation showed some effective bindings via spiroisoxazolin scaffold with the active site of COX-2 enzyme therefore we can expect COX-2 inhibitory activity for these compounds. Furthermore MTT assay proved our suggestion about antiproliferative effect of these proposal selective COX-2 inhibitors compounds. So designing these kinds of spiro compounds might be helpful in finding novel classes of potent anticancer agents.

Keywords: COX-2 inhibitor, MTT assay, Spiroisoxazolin, Docking
Title:
Investigation of azoospermic factor regions (AZFc and AZFd) microdeletions among infertile men with nonobstructive azoospermia from North West of Iran

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Abstract:
Introduction: The Y-chromosome azoospermic factor (AZF) regions include genes whose specific roles and functions in spermatogenesis have not been completely clarified. Hence, recognition of the association of AZF microdeletions with male infertility has suggestions for the diagnosis, treatment, and genetic counseling among infertile patients. In this work, we determined the incidence of Y chromosome AZF regions microdeletions in infertile men with non-obstructive azoospermic. Material and Methods: The descriptive -analytical study was performed between January 2012 and October 2012 on 47 infertile men with non-obstructive azoospermic and normal karyotypes referred to Infertility Center of Alzahra Hospital of Tabriz. Molecular AZF screening technique was performed on the genomic DNA from peripheral blood samples. We used Multiplex PCR and three different sets of sequence-tagged sites (STS) for detecting the microdeletions in Y-chromosomal AZF regions and the Y specific sequences. Statistical analysis was evaluated by Statistical software (SPSS, Chicago, IL, USA) version 11.2. Results: Among the 47 infertile men, a total of 17 cases (17/47, 36.17%) were found to have deletions in the regions of AZFc and AZFd. Of the 17 azoospermic subjects harbouring Y chromosome microdeletions, fourteen in AZFc, two in AZFc+d and one in AZFd regions. P value < 0.05 was considered to be statistically significant. Conclusion: From the results, Y chromosome microdeletions analysis recommend as an important molecular test among infertile males to obtain reliable genetic information before the administration of assisted reproductive techniques and will help decrease the cost and technical difficulty of the procedure.

Keywords: azoospermic factor, STS, spermatogenesis, microdeletions, male infertility
Title:
Antagonistic effect of combination therapy Trichostatin A and Etoposide on lung cancer H1299 cell line

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Abstract:
Introduction: Etoposide is used as chemotherapy for lung cancer. Whereas it have been largely unsuccessful in preventing relapse. Trichostatin A (TSA) as a histone deacetylase inhibitors have been extensively studied as potential candidates for treatment of various malignancies. The aim of this study was to investigate the combination effect of TSA on Etoposide-induced cytotoxicity in lung cancer H1299 cell line.

Material and Methods : H1299 cells were cultured; viability and cytotoxicity were assessed by Trypan blue and MTT assay. H1299 cell line was treated with 800nM Etoposide and 700nM TSA alone or combination of different dilution (4x, 2x, x, 1/2x, 1/4x) of them in time period of 72h. After addition of MTT solution on cells, OD wells were read using a spectrophotometer at 570nm wavelength.

Result: According to MTT assay results, treated of H1299 cells with TSA or Etoposide alone significantly induced cytotoxicity. But combination of these drugs did not show cytotoxic effect on cells (Cl<1).

Conclusion : Despite the positive effects of the mentioned drugs alone on cytotoxicity, not only in the combination of TSA and Etoposide was not observed cytotoxic effects on H1299 cell line but also antagonistic effects of them was seen. Therefore, it is suggested that probably TSA has different effect on different cancers and more studies should be carried out to elucidate the exact effects of TSA on in vitro models cancer.

Keywords: Trichostatin A (TSA), Combination Index (CI), Etoposide, MTT assay *x= (IC50 TSA+IC50 Etoposide)
Title: Comparative study of Trichostatin A and Carboplatin on ovarian cancer cell line growth

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Abstract: Introduction: Despite advances in radical surgery and chemotherapy, ovarian cancer is the most lethal of gynecological malignancy. Resistance to standard chemotherapy (carboplatin and paclitaxel) is one of the leading causes of therapeutic failure in ovarian cancer. Histone deacetylase inhibitor trichostatin A (TSA) represent a promising new class of anticancer agents. Trichostatin A has been shown to decrease cell survival, which suggests that HDAC inhibitors may be developed for preventing and treating ovarian cancer. In this study, we examined the Comparative cytotoxicity effect of trichostatin A and carboplatin on the growth of human epithelial ovarian carcinoma cell line (skov-3). Material and Methods: Ovarian cancer cell line (skov-3) was used as a model to investigate the cytotoxicity effects of different concentrations of carboplatin (20, 30, 40, 50, 60, 70, and 80 µM) and trichostatin A (200, 300, 400, 500, 600, 700, and 800 nM) after 48h treatment. The cytotoxicity effects of different concentrations of these drugs were evaluated by MTT assay. Result: The MTT result showed that IC50 for trichostatin A and carboplatin after 48 hours of treatment were 63 µM and 700 nM respectively. According to the result, trichostatin A and carboplatin affects the growth of Sk-ov-3 cell line and these cytotoxic effects were dose dependent manner. The nanomolar concentrations of TSA are effective. Conclusion: Based on obtained result, it is speculated that trichostatin A may have promise to become new therapeutic agents against ovarian cancer.

Keywords: Ovarian cancer, Trichostatin A, Carboplatin, MTT assay
Title:
Structure-based HMM matrix coefficients analysis of GPCR transmembrane helices

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Abstract:
Introduction: Advances in determining the structure of GPCRs can be a means of analyzing the distributions of amino acids in several regions of GPCRs including the intracellular and extracellular tail regions along with the transmembrane helical segments. Materials and Methods: The periodic distribution of amino acids in the sequence of 28987 TM GPCRs extracted from Uniprot has been analyzed with emission matrix probability values obtained from the hidden Markov model. The method does not involve any information about the physicochemical properties of amino acids. The illustrations obtained for six regions including the intra-/extra-cellular tail regions and helical segments showed the distribution of various classes of amino acids (i.e., aliphatic, hydroxyl or sulfur containing, cyclic, aromatic, basic and acidic and their amide). Results: The results showed that the aliphatic class and large hydrophobic amino acids are mostly seen in TM helical segments whereas the aromatic and charged amino acids are most prevalent in regions other than transmembrane regions. Moreover, basic and acidic and their amide amino acids were mostly seen in extra- cellular tail and intra-/extra- cellular tail regions respectively. The hydroxyl class types were seen mostly in even TM segments and the tail regions. The notable result was the presence of Arg, His, Lys that is for making cystolic ends of TM helical segments based on positive inside rule. Conclusion: The results obtained from the emission matrix probability values extracted from the Uniprot database strongly confirm the distribution of membrane proteins that can be also applied to GPCRs.

Keywords: Amino acid distribution HMM based analysis, GPCR proteins, Transmembrane helices
Title:
Nanodetection and Nanodrug delivery in lung cancer

Authors:
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Abstract:
Lung carcinoma is the most widespread type of cancer in the worldwide and is responsible for more deaths than other types of cancer. Each year the death from lung cancer is greater than the breast, prostate and colorectal cancer composed. Nanotechnology is the excited multidisciplinary field that contain the design and engineering of nano objects or nanotools with diameter less than 500 nanometers (nm) and it is one of the most interesting fields in the 21st century. Nanotechnology also offers the ability to detect diseases, such as tumor, much earlier than ever imaginable. This article presents Nano devices for lung cancer detection and drug delivery system.

Keywords: Nanodevice, Lung Cancer, Drug Delivery, Quantum Dots, Cancer, Detection
Title:
Validating signature genes in acute and chronic myeloid leukaemia in human bone marrow

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Abstract:
BackgroundCancer subtype classification using microarray signatures has the potential to transform pathological diagnosis but measurement of Indicator genes in routine practice remains difficult. We have previously used real-time PCR measurement of Indicator genes for acute lymphoid leukaemia (ALL) and acute myeloid leukaemia (AML) as a method for application of microarray gene signatures. The specificity of these genes for this distinction was tested by their measurement in patients with, chronic myeloid leukaemia (CML) and normal bone marrow. MethodsMononuclear cells were sorted into unselected (total), CD34+ve and CD34-ve fractions, mRNA globally amplified using PolyA PCR and the expression of 17 Indicator genes measured by real-time PCR. ResultsThere was no statistically significant difference in expression for any gene between cases of CML. Cyclin D3 only (p ≤ 0.04) was upregulated in CML in the CD34+ fraction, whilst HkrT-1 (p ≤ 0.02) and fumarylacetoacetate (p ≤ 0.03) were upregulated in AML. HOXA9 showed non-significant upregulation in AML, but in combination with proteoglycan 1 distinguished between AML and normal samples, in the CD34- fraction, in unsupervised clustering. Unsupervised clustering distinguished between AML and the other diagnostic groups. ConclusionThese results show that the genes discriminatory between ALL and AML are uninformative in the context of CML and normal bone marrow, except for distinction with AML.

Keywords: Microarray, PolyA PCR, RT-PCR, Gene Signature, Myeloid Leukaemia
Title:
Cell-free Protein Synthesis

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Abstract:
Introduction: Proteomics and biotechnology fields need simple and rapid methods to convert the genetic data into proteins. While recombinant protein expression in living cells is a time-consuming procedure, in vitro translation directs protein synthesis in hours from added linear PCR DNA without the requirement for a vector and cloning process. In addition, the open nature of cell-free protein synthesis (CFPS) and accessibility various prokaryotic and eukaryotic cellular lysates offer a flexible choice of conditions for production of "difficult" proteins that are not possible by in vivo systems. Several advantages of cell-free systems over common cell-based expression methods include the easy modification of reaction conditions to desirable protein folding, decreased sensitivity to product toxicity and suitability for high-throughput strategies. Recent developments in cell-free protein expression causenovel applications in biotechnology, proteomics and fundamental biological studies.Conclusion: Recently CFPS yields exceed grams of protein produced per liter reaction volume, costs have been decreased and reaction scale has reached the 100-liter milestone. Some challenges that remain in this field consist of synthesizing any biologically active protein reliably in a universal platform, lacking a cost-effective and scalable eukaryotic CFPS platform and inability to creating proteins containing glycosylation patterns resembling human profile. But we expected that this trend will continue and that CFPS will be a true alternative to cell-based protein expression systems.

Keywords: Cell-free protein synthesis, High-throughput methods, Protein synthesis, Protein engineering
Title:
Lab on a chip

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Abstract:
Lab on a chip (LOC) is a device that integrates one or several laboratory functions on a single chip of only millimeters to a few square centimeters in size, and nowadays because of unique properties of LOCs, they are more interesting for researcher. LOC technology may soon become an important part of efforts to improve global health, particularly through the development of point-of-care testing devices and many researchers believe that LOC technology may be the key to powerful new diagnostic instruments in this developing countries with few healthcare resources. LOCs may provide advantages such as: 1- LOCs may provide advantages, 2- faster analysis and response times, 3- better process control, 4- compactness of the systems, 5- massive parallelization, 6- lower fabrication costs, 7- safer platform for chemical, 8- radioactive or biological studies, 9- it is portable, ect. For creation of this device, Biotechnology and Nanotechnology must be integrated to help to fabrication of LOCs.

Keywords: lab on achip(LOC)
Title:
Functional bioactive peptides obtained from camel milk proteins

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Abstract:
There is a famous saying “Let food be your medicine and medicine be your food”. Currently there are a lot of interests in searching for functional foods that play a major part in improvement of human health. Nowadays an increasing attention is being focused toward consumption of camel milk. Its composition is closer to human milk than cow’s milk therefore its consumption is better for human especially for infant and children. Camel milk also contains antibodies that may help fight serious diseases like cancer, HIV/AIDS, Alzheimer’s and hepatitis B. It has been reported that multiple biologically active (bioactive) proteins and peptides can originate from milk. Bioactive peptides are a great source of natural drugs, which can both prevent and cure different diseases. These peptides can be produced in vivo during gastrointestinal digestion or in vitro through food processing using specific enzymes. Camel milk contains bioactive peptides with different biological functionalities. We have studied the functionality of the bioactive peptides produced from camel milk protein. The results of our studies showed that the bioactive peptides derived from camel milk protein had high functionality including antioxidant activity, anti-hypertension effect and antimicrobial activity. The bioactive peptides derived from camel milk protein had high functionality which was comparable to the commercially available drugs. Considering the health effect of camel milk proteins and its bioactive peptides and the fact that so many people could benefit from the healing properties of this milk, it could be introduced as a functional food.

Keywords: camel milk proteins, bioactive peptides, functional food, antioxidants, hypotension
Title:
Presence and Polymorphism of SopE Gene in Strains of Salmonella Typhimurium and Salmonella Enteritidis by PCR

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Abstract:
Introduction: SopE is a translocated effector protein involved in actin cytoskeletal rearrangements and membrane ruffling. As a virulence gene frequently transferred by bacteriophages, sopE gene is encoded in SPI-1, has been identified in isolates involved in major epidemics and plays a key role in emergence of epidemic strains. The study objective was to evaluate the polymorphism of sopE gene among type strains and clinical isolates of S. Typhimurium and S. Enteritidis. Method: A PCR reaction was developed to amplify sopE gene in type strains (S. Typhimurium LT2 and S. Enteritidis 147) and 14 previously confirmed clinical isolates (bovine sources) of S. Typhimurium and S. Enteritidis. Results: PCR products with four different sizes were observed: 300bp in 1 S. Typhimurium field isolate, 600bp in SE147, LT2 and 3 S. Typhimurium field isolates, 450bp in 2 S. Typhimurium and 2 S. Enteritidis field isolates, 900bp in 3 S. Enteritidis field isolates. 1 S. Typhimurium and 1 S. Enteritidis field isolates did not give any product with the applied PCR reaction. Conclusions: Previous studies have reported some polymorphism in sopE gene and proposed its application in serotype differentiation, but the present study showed a very much wider range of polymorphism among isolates of S. Enteritidis and S. Typhimurium. Further studies are undergoing to combine the polymorphism of this gene and some other bacteriophage-transferred genes to be applied in the phylogeny and source determination of strains responsible for clinical epidemic spread of the disease.

Keywords: SopE, Polymorphism, Salmonella, PCR