pole of the dimer, whereas the other pole carries domain swap mutations, which prevent binding to receptor. As HD-VEGF can only bind to monomeric receptors, it does not lead to signal transduction. Thus, HD-VEGF blocks KDR-mediated VEGF activities that are crucial in the angiogenic process and is therefore a promising, multipotent compound in the treatment of angiogenesis-related diseases. The receptor binding domain of wild type VEGF with N-terminal His-tag was expressed as inclusion body in E. coli and refolded successfully in our lab.To construct the heterodimeric VEGF variant, the precise KDR binding sites were determined from the crystal structure of VEGF-KDR complex (PDB ID: 3V2A). Considering to the φ and ψ dihedral angles, the distance between the first and the last amino acids and the length of these segments, some sequence with equall length and distance compared to those of VEGF were searched in pdb site. The binding sites in VEGF crystal structure were replaced by suitable sequences. The model of designed mutant VEGF was compared to native one after energy minimization process. Based on constructed model, the receptor binding domain of mutant VEGF gene was designed, synthesized and inserted in pET-21a expression vector with additional N-terminal Strep-tag which providing a system that able to purification of heterodimeric from homodimeric variants by two affinity chromatography. The mutant VEGF was expressed in E. coli and after purification of heterodimeric variant, its anti angiogenic property will be investigated in future.

Keywords: Angiogenesis, Antagonistic VEGF, Heterodimer, Strep-tag.

Abstract No.187

ZnO and CuO Nanoparticles Increase Extracellular Glutamate Concentration in Synaptosomes of Rat Brain

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In modern life, human beings are exposed to nanoparticles from ambient air and certain work places. There have been increasing reports that inhaled nanoparticles can reach the brain through two main routes: blood circulation and olfactory bulb penetration. More over exposure to nanoparticles in vivo increases the risk of neurodegenerative diseases while in vitro studies show that nanoparticles can kill neurons. How ever the mechanisms underlying this phenomenon are still unclear. It is believed that the toxicity of nanoparticles in brain is mediated by induction of oxidative stress in microglia cells and astrocytes. However neuronal presynaptic terminals could be other targets for nanoparticles due to high turn over of synaptic vesicles in these terminals. In this case it has been shown that ferritin, as an iron nanoparticle, inhibits glutamate uptake. Glutamate is the major excitatory neurotransmitter in mammalian central nervous system. Excessive extracellular concentrations of this neurotransmitter could cause hyper activation of glutamate receptors and result in cell death. In the present work the effect of ZnO and CuO, two metal oxide nanoparticles that are widely used in industry, has been studied on the extracellular concentration of glutamate in rat Synaptosomes. We found that ZnO and CuO nanoparticles can cause remarkable increase in extracellular glutamate in Synaptosomes. We suppose that glutamate uptake is inhibited in the presence of these metal oxide nanoparticles.

Keywords: Synaptosomes, Neurodegenerative Diseases, ZnO/CuO Nanoparticles.

Abstract No.188

Determination of Microscopic Dissociation Constants of Tryptophan

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The study of the interactions between surfactants and proteins is of significant scientific and technological importance [1]. The macroconstant pKa values are commonly known while the microconstant values (especially tautomerization constant Kz) are often not recognized [2]. The concept of microscopic constants is important and interesting, and it has the advantage of being directly related to the specific functional groups involved, thereby, enabling investigators to interpret such parameters in terms of molecular structure. Determination of microscopic constants and tautomeric ratios has played an important part in understanding the ionic composition of many biologically active molecules, particularly because all proteins fall into this class. Furthermore, the microscopic constants and tautomeric ratio describe the amount of various species as a function of pH [3]. The present work is an attempt to study the effect of anionic surfactant SDS on the dissociation equilibria of the amino acid tryptophan. In this study, we have determined macroconstant Ka1, Ka2 values, tautomerization constant kz and microconstants k11, k12,

k21, k22 values for the Tryptophan aqueous solution and tryptophan solutions in the presence of different concentrations of SDS.

Keywords: Microscopic Dissociation Constants, Tautomeric Ratios, Tryptophan, Sodium Dodecyl Sulfate.

Abstract No.189

Probing Crucial Amino Acids Role in Chondroitinase ABCI Enzyme Activity by Site-directed Mutagenesis

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Chondroitinase ABC I is an112.5kDa enzyme with 997 amino acid .This enzyme is a GalAG (galactosaminoglycan) depolymerizing lyase with a broad-specificity and depolymerizes a variety of GAG substrates,including C4S (chondroitin 4-sulphate), DS (dermatan sulphate), C6S (chondroitin 6-sulphate) and hyaluronic acid. Previous studies suggested that cABC1 enzyme promote neural system regeneration by degrading GAG chains followed by decreasing CSPG s inhibitory effect so there is a wide range of application of the enzyme in medicine .cloning and expression of the enzyme have been done successfully in BL-21. Three Dimensional structure analysis with x-ray crystallography suggested that the enzyme has three major domains. The optimum pH and temperature in Tris buffer is pH=8 and 37 °C, respectively . In order to increase thermal stability we applied quik change mutagenesis. We use a thermophile strain thetaiotaomicron WAL2926 as a template to guide us the best selection of amino acids for enzyme engineering. In this study, proline 485 has been substituted with alanine. finally the activity and stability of the wild type and its varint has been discussed and proven.

Keywords: Chondroitinase ABC I, Galactosaminoglycan, Site Directed Mutagenesis Stability.

Abstract No.190

Monoclonal Antibodies in the Treatment of many Diseases in Mice Experimentally Using Veterinary Clinic of Sina Gilan,Iran

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Monoclonal antibodies(MAB)have found increasing use experimental therapies.great limitation of their use is that they are recognized by the patient as being of foreign origin and an antiglobulin response is provoked.recombinant DNA technology offers the ability to convert these rodent antibodies into a more human form.there are currently several different strategies which can be adopted to generate humanized antibodies resulting in different degrees of humanization can be achieved ranging from chimeric antibodies with a combination of human constant regions with rodent variable regions to fully reshaped antibodies where the variable regions are also humanized.At present the available data on clinical use of chimeric and reshaped antibodies is very limited. The rat IgG2b antibody CAMPATH-1G has been shown to be both useful as an immunosuppressive antibody as well as in the treatment of lymphoid malignancies . The reshaped version, CAMPATH-1H, was successfully used to clear detectable malignant cells from the blood and bone marrow in two patients with B-cell lymphoma . A more sustained course of the human antibody (126 mg over 30 days and 86 mg over 43 days) was tolerated than had previously been used for the rat antibody.In the future in veterinary clinic of Sina gilan, it is clear that a majority of monoclonal antibodies produced for therapy will be humanised for the reasons discussed above. As far as improvements in the abilities of these antibodies to interact with human effector mechanisms goes it seems that there is unlikely to be any major differences between chimeric and fully reshaped antibodies.important about humanised antibodies is whether there are any sequences contained within the variable region frameworks, complimentary determining regions or constant region allotypes which can be processed and presented as T-cell epitopes.

Keywords: Monoclonal Antibody, Chimeric Antibody, Recombinant DNA Technology, Humanized Antibody, Antiglobulin.
