bacterium was isolated from fruit waste soil based on the potential degradation of the highly methylated pectin. According to the 16S rDNA sequence analysis, it was identified as Bacillus licheniformis BR1 which produces an extracellular polymethyl galacturonase PMG (EC 3.2.1.64) when cultured in medium containing citrus pectin0.1 %, peptone1%, yeast extract1%, pH 7.0. PMG production was optimized (6.22 U/ml) and the enzyme was purified to homogeneity in two steps column chromatography. The enzyme was a dimeric protein and exhibited an apparent molecular mass of 104 kDa by gel filtration chromatography and 56 kDa while running on SDS-PAGE. The enzyme exhibited maximum activity at 60 °C, pH 6 with 49% of total activity at 90 °C for 30 minutes and extends range of pH stability (5.0-9.0). Kmand Vmax of the PMG were determined 0.066 µmol/min and 2.51mg/ml respectively, using pectin as substrate. PMG activity significantly enhanced in the presence of 5 mM Ca2+, Cu2+, Mg2+, Mn2+ and Co2+, although Hg2+ and Fe2+ served as strong inhibitor. The enzyme was identified as a metal-dependent pectinase, which was inhibited by 5 mM of EDTA and showed suitable stability in the presence of 0.1% SDS and Tween 80. In overall, regarding to acidic properties and high operational stability of the purified pectinase, it seems that PMG can be an ideal functional substitute for applications in fruit juice industry, especially in citrus fruits extraction and clarification.

Keywords: Pectinase, Bacillus licheniformis, Purification, Polymethyl galacturonase, Thermostablity.

Abstract No.185

Effect of Chemical Modification on Thermal Stability, Structure and Function of Horseradish Peroxidase

Rasool Nowruzi, *Leila Hasani**

Department of Biological sciences, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan, IR
(E-mail: rasool.nowruzi@yahoo.com)

Biocatalysts are increasingly employed in chemical processing because of their selectivity as well as their potential as a greener alternative to chemical catalysis but, they are not usually tolerant to the presence of organic solvents, extremes of pH or high temperatures. Consequently, there is great interest in developing strategies to improve protein stability in desired reaction media. Compared with the strategies for obtaining stable enzymes, chemical modification is a simple and effective technique. Horseradish peroxidase (HRP) has achieved a prominent position in the pharmaceutical, chemical, and biotechnological industries therefore, methods improving the stability and functionality of HRP will clearly broaden the range of its present and future applications. In the present study, chemical modification of HRP was carried out with 2,3-dichloromaleic anhydride and 2,3-dimethylmaleic anhydride. Thermoinactivation, kinetic parameters and activation energy of catalysis and structural changes of HRP upon the modification were studied using spectroscopic techniques. The results indicated that 2,3-dichloromaleic anhydride increases thermal stability of HRP but 2,3-dimethylmaleic is not a stabilizer modifier for HRP. Catalytic efficiency and activation energy did not change remarkably following reaction of the enzyme with the carboxylic anhydrides. Fluoresnce measurements indicated that the intensity of protein emission decreases slightly upon the modification with both modifiers. A decrease in the distance between the heme and the tryptophan residue or decrease in compactness of the structure and therefore exposure of trp residue to solvent may lead to this change. On the whole, comparing the effect of 2,3-dichloromaleic with 2,3-dimethylmaleic implies that hydrophilization of the protein surface results in thermal stability of the protein.

Keywords: Horseradish Peroxidase, Thermal Stability, Chemical Modification, Spectroscopy.

Abstract No.186

Design and Construction of Antagonistic VEGF Variant for Inhibition of Angiogenesis

Fahimeh Ghavami [1], Shirin Shahangian [1], Reza H. Sajedi [2], Shahriar Arab [3], Mahmoodreza Aghamaali [1], Kamran Mansoori [4]*

1. Department. of Biology, Faculty of Science, University of Guilan, Rasht, IR
2. Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, IR
3. Department of Biophysics, Faculty of Science, Tarbiat Modarres University, Tehran, IR
4. Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, IR
(E-mail: ghavami.fahimeh@yahoo.com)

Because of its central role in pathological angiogenesis, vascular endothelial growth factor (VEGF) has become a major target for anti-angiogenic therapies. We report here the construction of a heterodimeric antagonistic VEGF variant (HD-VEGF). In this antagonist, binding domains for the VEGF-receptor KDR/Flk-1 is present at one

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

Shahrbanoo Rafiei Taghanaki, *Gholamhossein Riazi**, *Shahin Ahmadian*

Department of Biochemistry, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, IR
(E-mail: l.rafiei79@gmail.com)

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

*Marjan Farhangi**, *Mohammad Reza Housaindokht*, *Narjes Ashraf*

Ferdowsi University of Mashhad, Mashhad, IR
(E-mail: Housain@um.ac.ir)

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,