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Calcium has a crucial role in activity and stability of amylases, all amylases have at least one Ca2+ per molecule, therefore specific and conserved amino acids are involved in calcium binding. In the present study, sequence analysis revealed the presence of EF-hand like motif in calcium binding loop of BMW-amylase that was previously isolated from Bacillus megaterium WHO. The EF-hand motif and its variants (EF hand like motif) are the most common calcium-binding motifs that found in various calcium binding proteins. We used site directed mutagenesis to replace His 58 with Asp (D), Ile (I), Tyr (Y), Phe (F) and Arg (R) at the seventh position of EF hand like motif to investigate the effect of calcium ion on the thermal stability, activity and structure of BMW-amylase. Effect of calcium on wild type and its variants, that monitored by fluorescence techniques, showed no significant changes. All mutants retained their hydrolytic activity and upon the addition of an extra DX unit to the calcium binding loop in H58D variant, thermal stability, catalytic activity and chelating power of the enzyme improved due to higher affinity toward calcium.

Keywords: EF-hand Like Motif, Calcium Binding Proteins, DX unit, BMW-Amylase, Chelating Power.

Abstract No.82

Evaluation of Metal-Ion Affinity Enrichment of Phosphoprotein

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Phosphorylation and dephosphorylation of cellular proteins play a fine tuning function during signal transduction that affect cellular processing and survival of living organism as a whole. Phosphoprotein profiling, thus, is an active research area to understand the molecular basic of cellular activities, but is hampered because of the low abundance of cellular phosphoproteins. Hence, enrichment of cellular phosphoprotein was highly recommended. Currently, three techniques: (I) immobilized metal ions affinity electrophoresis (IMAEP), (II) immobilized metal ion affinity chromatography (IMAC), and (III) immobilized metal ion affinity nanoscale magnetic beads (IMANMB) were used for phosphoprotein enrichment. These techniques share common principle, i.e., phosphoproteins are captured through interaction with metal ions that in turn is immobilized to inert matrix. Therefore, we have analyzed and compared the three techniques using purified phosphoproteins and cellular phosphoproteins. Comparison with IMAC (QIAGEN) shows that both IMAEP and IMANMB (Clontech) could be carried out with small sample size. In contrast, the enrichment duration was of following order: IMANMB < IMAC < IMAEP. The sensitivity with regard to sample size was nearly same between IMAEP and IMANMB but less in IMAC. However, using of lab synthesized IMANMB need to be undergoes thorough examination for its applicability compared with commercially available IMANMB. For example, in our recent case, IMANMB supplied by chemistry department, IASBS, shows the presence of non-phosphoprotein contaminants. This is not the case with Clontech IMANMB under similar condition of enrichment process. This contaminant seems to be mainly come from porosity and thickness of the outer coating layer of magnetic particles. The less removal of contaminants even after several washing with binding buffer and removal of the same along with the phosphoprotein with elution buffer clearly indicate that this non-specific binding is also electrostatically entrapped with either core/shell or central magnetic core.

Keywords: Phosphoprotein, Phospho Magnetic Beads, IMAEP, IMAC.

Abstract No.83

Bioinformatics Analysis of Helicobacter Pylori IceA, an Ulcer-associated Gene Restriction Endonuclease (nlaIIIR)

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Helicobacter pylori, which colonizes the gastric mucosa of human, plays an important role in development of peptic and duodenal ulcers as well as gastric cancer. The iceA, a member of the virulence genes of the bacterium, represents two distinct genotypes, iceA1 and iceA2. The iceA1 gene reveals strong homology to a restriction endonuclease (nlaIIIR) of Neisseria lactamica. This is relevant clinically because the strains harboring iceA1 genotype are associated with peptic ulcer disease (PUD) and increases acute neutrophilic infiltration. The purpose of this study was to produce the theoretical three-dimensional structure of IceA1 and IceA2 in order to perform a comparative structural analysis of two forms of the protein. The models were constructed using comparative and fold recognition modeling methods

and the stereochemical quality of protein structures and molecular dynamics simulation of the refined models was performed. The structural analysis showed the key features of IceA1 relative to IceA2 that may illustrate their different potentiality for interaction with possible ligands or inhibitors.

Keywords: Helicobacter Pylori, IceA, Peptic Ulcer Disease, Comparative Modeling.

Abstract No.84

Suicide Inactivation of Horseradish Peroxidase by Aminophenol Using Voltametric Method

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Horseradish peroxidase (HRP) is the enzyme which catalysis the oxidation of variety of molecules in the presence of hydrogen peroxide (H_2O_2) and used as a label in biosensors. In such biosensors, HRP detect the biological reaction (e.g. antigen-antibody), which essentially is applicable for determination of desired analyte. The use of peroxidase substantially increases the sensitivity of this method.In this report, we studied the reaction of HRP with o-aminophenol (o-AP) as a substrate. The oxidation of o-AP is owing to the presence of two functional groups, OH and NH_2, which undergo hydrolysis of o-AP and produce the reactive soluble intermediates such as 0-quinone imine. Finally this intermediate is changed into 2-aminophenoxazine-3-one. In order to identify the effect of o-AP on inactivation of peroxidase, we used cyclic voltammetry and glassy carbon as working electrode. After optimization of the results, it was revealed that to get the maximum efficiency of enzyme, the optimum concentration for H_2O_2 ando-AP must be less than 10 and 2 mM, respectively.

Keywords: Horseradish peroxidase, Aminophenol, Hydrogen peroxide, Cyclic Voltammetry.

Abstract No.85

Protein Structure and Function in Different Surface Nanoparticle: Applications in Biological Area and Biomedicine

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The conformational changes of glucose oxidase (GOx) in the negative charge colloidal gold nanoparticles (NC-GNPs), positive charge colloidal gold nanoparticles (PC-GNPs), mercaptopurine:GNPs and 11-mercaptoundecanoic acid:GNPs as a hydrophobic and hydrophilic GNPs was investigated by various spectroscopic techniques including UV-Vis absorption, fluorescence and circular dichroism (CD) spectroscopies. Moreover, the fluorescence quenching constant and binding parameters after the formation of the GOx:GNPs conjugates follows by Stern-Volmer (S-V) plots. Size and charge of NC-GNPs and PC-GNPs was determined by zeta sizer and zeta potential analyzer, which their size and charge are 83.7 nm (-16.4 mV) and 90.4 nm (+8.44 mV) respectively. Also GOx activity and amount of protein adsorption on GNPs was determined. The results showed that NC-GNPs have least conformational changes for GOx at both secondary and tertiary structure levels. And hydrophobic-GNPs with the minimum protein adsorption have a maximum conformational change

Keywords: Negative Charge Colloidal Gold Nanoparticles, Positive Charge, CD Spectroscopy, Glucose Oxidase.

Abstract No.86

Interaction Studies Between a [(phen)Pd µ-(S2CNH(CH2)3NHCS2)Pd(phen)](NO3)2 anti-Tumor Complex and Calf Thymus DNA

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Palladium(II) complexes are very interesting candidates for alternative platinum-based drugs, because the coordination geometry and complex forming processes of palladium(II) are very similar to those of platinum(II).Thus we focused on the interaction of calf thymus DNA (CT-DNA)with a new palladium(II) anticancer complex offormula [(phen)Pd µ-(S2CNH(CH2)3NHCS2)Pd(phen)](NO3)2 (where phen is 1,10-phenanthroline and S2CNH(CH2)3NHCS2is propylenbisdithiocarbamato).This interaction was studied by spectroscopic techniques such as UV-Vis and fluorescence in 30mM