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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