from Bacillus thermoproteolyticus, which is thermostable at temperatures above 80 °C, and elastase from Pseudomonas aeruginosa, which is highly stable/active at organic solvents was designed and constructed. The extended surface region with 46 residues was replaced with the equivalent region from thermolysin containing 52 residues. Accordingly, the chimeric enzyme was expressed , purified and characterized against elastase for caseinolytic activity inpresence of varying concentrations (V/V%) of organic solvents including ethanol , methanol, n,n Dimethyl formamide (DMF), ethylene glycol and isopropanol. Although both enzymes were highly active in all organic solvents , elastase was more active than chimer in ethanol while chimer was more active in ethylene glycol. However , activities were almost similar in other organic solvents DMF, methanol and isopropanol. Finally, it may conclude that the engineered surface region is not responsible for organic solvent stability/ activity of elastase .

Keywords: Protein Engineering, Zn- Metalo Proteases, Elastase, Extended Surface Region, Organic Solvent Activity.

Abstract No.154

Reconsidering the Anti-Glycation Activity of Acetylsalicylate on Bovine Serum Albumin

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Glycation is consequence of covalent bonding of reducing sugars to proteins or lipids without assistance of an enzyme which is ultimately bring about formation of advanced glycation end-products (AGEs). This process is confirmed to be associated with several pathophysiological disorders such as neuro-, retino- and nephro-pathies.Also, t has been suggested with a fortiori that acetylsalicylate (ASA) acts as an anti-glycation agent through acetylation of amino groups although, the exact mechanism remains problematic and unclear. In this study, glycation of bovine serum albumin (BSA) with fructose was assessed in which the interference ofASA and several rationally related compounds including benzoic acid was analyzed by various methods such as UV-Vis, fluorescence and circular dichroism spectroscopies. As a result, salicylation instead of acetylation are proposed as a dominant mechanism of inhibitory effect of ASA on glycation process.

Keywords: BSA, Acetylsalicylate, Glycation, Acetylation, Advanced Glycation end Products (AGE).

Abstract No.155

Evaluation of Molecular Mechanism Associated with Reversibly Arrested Zebrafish (D. rerio) Embryo Development Induced by Temperature

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Zebrafish embryos can undergo a reversible metabolic and developmental arrest termed diapauses during unfavorable conditions. Diapause-I that occurs early in development before the establishment of the embryonic axis, can be induced by lowering embryo incubation temperature from 28.5 oC normal temperature (NT) to 15±1°C. We have investigated the molecular mechanism of diapause-I during Zebrafish embryo development using various techniques. When diapause-I was induced at 15±1°C for 3 hours, it could be fully recovered at normal developmental speed after returning to NT. But if diapause-I was induced at 4°C, after returning to NT, the recovery speed was much slower and most of the embryo did not survive. Using Comet assay, we found that induced diapause-I at both 15±1°C and 4°C, were not associated with embryonic apoptosis. However, quantification of embryonic DNA showed that increases DNA content in diapause-I at 15±1°C but not in case of diapause-I at 4°C compared to the DNA content before arrest. Diapause-I at 15±1°C thus suggests, this embryo was active physiologically, metabolically and can undergo DNA replication but in a slower speed. However, lack of morphological changes during diapause-I suggests inhibition of cell plate movement during diapauses-I. This was based on the finding that one prominent yolk protein of molecular weight at around 97 kDa was unaffected during diapause-I at 15±1°C, which otherwise, under normal condition undergoes continuous decrease with the increasing ages developing embryo. However, diapause-I at 4°C, the 97 kDa yolk protein was severely affected and was absence from the SDS-PAGE protein lane, if the SDS-PAGE was carried out with the non-denaturing detergent solubilized embryo sample but not with the denaturing detergent solubilized embryo sample. The results suggests Diapause-I at

15±1°C, 97 kDa yolk protein was highly available during recovery period at NT, but not in case of 4°C as this protein probably undergoes aggregation thus non-available for cell plate movement.

Keywords: Zebrafish, Embryonic Development, Temperature, Diapause.

Abstract No.156

Role of Low Molecular Weight Biomolecules in Antioxidant Profile of Camel and Bovine Milk

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As reported previously, the antioxidant activity of milk comes from both low- and high molecular weight biomolecules. In this study antioxidant activity of whole milk and milk protein fractions (WF: Whey Fraction, and CF: Casein Fraction) were examined, using an ABTS radical cation assay. To explore the effect of thermal stress on antioxidant activity of either whole milk or milk protein fractions (WF and CF), their antioxidant activities were measured before and after heat treatment for 5 min at 100 °C. The results of heat treatment experiments, suggest that antioxidant activities of both whole milk and WF of camel were more temperature labile than those from bovine source. Also, a slight reduction in antioxidant activity of CF of both animal sources was observed after the heat treatments. Moreover, prior to performing antioxidant measurements, whole milk and WF were extensively dialyzed. The objective was to explore role of low molecular weight (LMW) biomolecules in their antioxidant profile. The results showed that LMW biomolecules participate to the higher extent in shaping antioxidant profile of milk and WF of camel compared to those from bovine source. The antioxidant activity of LMW biomolecules was more heat labile than that of high molecular weight (HMW) biomolecules such as proteins. Overall, this study suggests a significant role for the heat labile LMW biomolecules in shaping the antioxidant profile of camel milk.

Keywords: Antioxidant Activity, Camel Milk, Bovine Milk, Low Molecular Weight Biomolecules, Heat Treatment.

Abstract No.157

Thermal Inactivation and Conformational Lock Study on Horse Liver Alcohol Dehydrogenase

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Horse Liver Alcohol Dehydrogenase (HLADH) is a two subunits metal enzyme that has two catalytic and coenzyme domains for each subunit. The catalytic domain contains two zinc atoms that one of them has a catalytic role and another one has a structural role. The coenzyme domain connects to NAD+ coenzyme for oxidoreductase reactions. These subunits were connected to each other by coenzyme domains. In this report, we are wondering to obtain the precise residues that participate in subunits via interface locks. For this purpose, the kinetics of thermal inactivation of HLADH were studied in a 50 mM pyrophosphate buffer, pH 8.8, using ethanol as substrate and NAD+ as a cofactor. The temperature range was between 46-55°C and the conformational lock was developed based on the Poltrak theory and analysis of the curves was done by the conformational lock method for oligomeric enzymes. We obtained the number of the locks between subunits equal to two by this method and then confirmed it by the Ligplot program computations. This computation give us more detailed information that there are two patches binding sites in the interface that they spread over two regions of each chain. The small region embraces the sequence of 110 to 118 residues and the large region contains residues of 270 to 320 on each chain. The first small and second large patches may be split to two smaller sub-patches.

Keywords: Horse Liver Alcohol Dehydrogenase, Conformational lock, Poltrak theory, Ligplot program.

Abstract No.158

Autolysis Comparison on Isoenzymes of Ficin Extracted from the Fig (Ficus carica cv. Sabz) Latex

Hamid Zare [1], Maryam Salami [1], Ali Akbar Saboury [1], Morteza Mirzaei [2], Farzaneh Farivar [1], Reza Hosseinzadeh [1], Ali Akbar Moosavi-Movahedi [1]*