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