Abstract No.151

The effect of Pomegranate Juice Supplementation on Oxidative Stress Following Exhaustive Exercise in Young Healthy Males

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Pomegranate fruit has potent antioxidant effects and may play a role in reduction of oxidative stress in healthy male athletes. Therefore, this study examined the effect of pomegranate juice supplementation on oxidative stress following exhaustive exercise in young healthy males.In an interventional study, 14 healthy male student athletes at 18 - 24 years old living in Dormitories of Ardabil University of Medical Sciences randomly were selected. After getting consent, general Information, anthropometric factors (height and weight), and fasting blood samples, one cup (240 ml) pomegranate juice supplementation were given to subjects per day for two weeks. Fasting blood samples were taken at the end of two weeks intervention. To subjects were given once exhaustive exercise and then so fasting blood samples were taken. Fasting blood samples were used for testing of serum MDA, total antioxidant capacity, glutathione, lipids profile, glutathione peroxides, superoxide dismutase, and paroxonase1 levels. Data were analyzed using descriptive statistical tests and Paired sample t-test.This study showed that the level of serum total antioxidant capacity and activities of enzymes such as superoxide dismutase, glutathione peroxidase, arylesterase, and standardized arylesterase activity were significantly increased following exhaustive exercise ($p<0.05$). The serum level of malondialdehyde was significantly decreased after exhaustive exercise ($p<0.05$). We observed no significant changes in serum levels of lipids profile, glutathione, and paroxonase1 activities after exhaustive exercise.Findings of this study suggests that the intake of pomegranate juice supplementation may be useful on body antioxidant defense system and reduction oxidative stress in exhaustive exercise.

Keywords: Pomegranate Juice, Oxidative Stress, Antioxidant, Exercise, Exhaustive.

Abstract No.152

Engineering a Pseudolysin to Resist Elevated Temperatures

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Neutral proteases (NPs) are zinc metalloproteases with biotechnological applications such as aspartame and peptide synthesis, but autolysis at high temperature is a limitation in this regard. Here, we replaced an extended surface region of elastase from Pseudomonas aeruginosa (pseudolysin), with single bound calcium, with the same region from thermolysin from Bacillus thermoproteolyticus, with three bound calcium to improve the thermostability of enzyme. Thermal stability of Zn-metalloproteases is quantified in terms of T50, the temperature for which a 30 min incubation reduces the enzyme activity by half. Accordingly, T50 was determined both enzymes. Moreover, the first order inactivation process was compared at different temperatures 55 to 90 °C. Based on the results, both T50 and $t_{1/2}$ remarkably improved for chimeric enzyme. To further assess the thermostability, thermodynamic parameters of inactivation process including activation energy (E_a), $\Delta H^{\#}$, $\Delta G^{\#}$ and $\Delta S^{\#}$ were calculated and compared. We concluded that the Ca-binding surface region is determinant for thermostability of pseudolysin.

Keywords: Pseudolysin, Autolysis, Stability, Protein Engineering.

Abstract No.153

Impact of an Extended Surface Region on the Organic Solvent Activity of Thermolysin Family

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Proteases are broadlyuse in peptide synthesis, protein processing, and food, pharmaceutical and detergent industries (Anwar and Saleemuddin, 1998, Gupta et al., 2002). They hydrolyze peptide bonds in aqueous while synthesize them in non-aqueous environments. As biocatalysts for peptide synthesis, proteases need to be stable in the presence of organic solvents. Here, a chimeric gene of thermolysin

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