responsible for lens transparency, beta and gamma crystallins possess mainly structural role. As reported previously, the interface of two domains in bovine gamma-B crystallin consisting many residues including Phe56 which is critical for both efficient domain pairing and protein stability. While slight destabilizing outcome has been reported for the Phe56Trp mutation, its replacement with either Ala or Asp resulted in drastic instability impact. In this study, Phe56 was in silico mutated for different residues in both bovine and human gamma-B crystallins and their effects on the protein stability was evaluated using different bioinformatics tools. Bovine gamma-B crystallin and its human counterpart demonstrate 83.9% homology as determined using clustalW software. The results show that all mutations performed at this position, depending on size, polarity and charge of substituted amino acid side chain, leading to range of protein instability in both human and bovine gamma-B crystallins. All substitutions performed resulted in significantly higher destabilizing consequence for human gamma-B crystallin compared to that of bovine protein counterpart, suggesting the impact of fine tertiary structures on stability of these proteins. Furthermore, our results indicated strong destabilizing properties for the substitution of Phe56 with the aliphatic residues of Gly, Ala, Val and Leu, and the degree of destabilization was strongly associated to the size of amino acid side chain. Overally, this study suggests that both polarity and size of amino acid side chains might be considered as the determining factors in fine complementary pairing of two domains which are required for both structural integrity and stability of gamma-B crystallin.

Keywords: Gamma-B crystallin, Stability, Mutation, Domain, Phe56.

Abstract No.138

Engineering of Solvent Exposed Loops of Firefly Luciferase Through Arginine Saturation

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Firefly luciferase (EC 1.13.12.7) catalyses the oxidation of aluciferin in the presence of magnesium ions, ATP and molecular oxygen. The product,oxyluciferin, is generated in an excited state which then decays tothe ground state with the emission of a photon.In life science, a wide range of novel invitro and invivo applications of Luciferases has been developed.However,several factors limit further application and development ofthis technology, including the low stability of the enzyme both invitro and in vivo,a low turnover number and a high Km for the substrate ATP. Protein engineering studies show that thermostable proteins have higher frequency of Arg, especially in exposed state, which would stabilize the exposed part of protein structure.According to bioinformatics analysishigher ratios of charged amino acids, especially at thesurface, increase ion interactions and enhance occurrence ofsalt bridges and ion pairs in thermophilic proteins whichprovide thermal stability to proteins.Here, we have extended this strategy by mutating severalhydrophobic residues scattered at the surface ofL.turkestanicus luciferase to arginine.After production of mutants luciferase,the native and mutants luciferase have overexpressed and purified. Thermostability and other properties were determined and results show that after 40 min incubation ofenzymes at 40°C, the relative remaining activity of wildtype was only 5%, whereas was formutants luciferaseswas 80% of original activity.Also, half-lives ($t_{1/2}$) of luciferase inactivation are measured and results show thatand mutants luciferasesare increased to 15 and 19.2 min,which is much higher than wild-type luciferase (2.6 min).We have shown that replacement of uncharged polar and hydrophobic residues by Arg leads to the enhancement of thermostability.

Keywords: Luciferase, Oxyluciferin, Hydrophobic Residues, Thermostability.

Abstract No.139

Tramadol Induces Inhibition and Structural Changes on Kidney Alkaline Phosphatase

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Alkaline phosphatase (ALP) is a hydrolytic enzyme with various type of substrate including proteins, nucleotides and phospholipids. ALP removes phosphate group from the substrate. Tramadol is a narcotic-like pain reliever and uses to treat moderate to severe pain. In this study the effect of tramadol on the alkaline phosphatase activity was investigated. The cell free extract was prepared from balb/c mouse kidney and used for enzyme assay. Our results showed that tramadol inhibited kidney alkaline phosphatase activity. The Km (0.28 mM) of enzyme did not change, while the Vmax of enzyme reduced in the presence of drug. The Ki and IC50 values of tramadol were determined

to be about 0.19mM and 0.09mM, respectively. The enzyme was purified after ammonium sulfate precipitation, DEAE cellulose and G100 gel filtration chromatography. SDS-PAGE electrophoresis showed only one bond with molecular weight of 6.5 kDa. The fluorescence emission of pure enzyme showed that tramadol could bind to both free enzyme and enzyme-substrate complex which was accompanied with reduction of emission intensity and conformational changes.

Keywords: Alkaline Phosphatase, Drug, Inhibition, Tramadol, Kidney.

Abstract No.140

Structure-function Studies of Three Types of Enzymes Involved in Disease, a Pectate Lyase, an E3 ubiquitin Ligase and Three Methyltransferases

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In the current world of research on agents of disease, proteins (enzymes in particular) are of great importance as they play a pivotal role in infection. Structural and functional studies of enzymes can be carried out through the use of biophysical and biochemical methods, in order to answer the many yet unanswered questions about their important roles.In this talk the results from the structural and functional studies carried out on two bacterial enzymes causing disease in plants and humans will be presented. These bacterial enzymes are pectate lyase1 from Bacillus subtilis and IpaH9.8 E3 ubiquitin ligase2 from Shigella flexneri which cause infection in plants and humans, respectively. Pectate lyase is involved in crop spoilage while IpaH9.8 E3 ubiquitin ligase is involved in the colonization of human intestinal cells causing Shigellosis, a severe bloody diarrhea. In addition, structural data on methyltransferases3 essential for the very important Vitamin B12 biosynthesis in Rhodobacter Capsulatus will be presented. It is important to mention that these methyltransferases play an important role in decorating tetrapyrroles in the Vitamin B12 biosynthetic pathway. Vitamin B12 produced by bacteria is an essential dietary requirement for humans and its deficiency can potentially cause severe and irreversible damage, especially to the brain and nervous system and is therefore of great medical importance.The biochemical techniques used in this study include protein over-production, protein purification, SDS-PAGE analysis, affinity chromatography, gel filtration chromatography, ubiquitination assays and western blot analysis. The biophysical techniques used include dynamic light scattering (DLS), isothermal titration calorimetry (ITC), crystallisation screening, and X-ray crystallography leading to structure determination.These techniques have come hand in hand in answering many questions relating to the structure and function of these enzymes of interest and have opened up new avenues in furthering our knowledge about these agents of disease.

Keywords: Disease, Pectate Lyase, E3 Ubiquitin Ligase, Methyltransferases, Crystallography.

Abstract No.141

Micro-/ Nanoparticles from Polyethylene Glycol and Poly(L-lactic acid) Triblock Copolymer in Aqueous Solution as Drug Delivery Systems

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Block copolymers are linear macromolecules that consist of two or more different blocks of different types of monomers. Amphiphilic block copolymers self-assemble in suitable solvents to form various supramolecular structures, like spherical and/or worm micelles, flower-like micelles, vesicles, microspheres, etc. Over the past decade, the effectiveness of such self-assembled structures as drug delivery vehicles has been demonstrated. In this research, different PLA-PEG-PLA (PLA is poly(L-lactic acid) and PEG is polyethylene glycol) triblock copolymers varying in block lengths are studied. The formation of micro-/ or nanoparticles made of these copolymers is investigated. The effect of hydrophilic / lipophilic balance (HLB) on the critical aggregation concentration (C.A.C.) and size distribution of the particles is reported. On the basis of obtained results, PLA37-PEG136-PLA37 was selected for other studies. A comparative study on the interaction of this copolymer with human and bovine insulins was carried out using CD, fluorescence and UV-Vis spectroscopy. Binding affinities of copolymer to both proteins are comparable: K_d(Bovine)= 29.17×10^{-6} M and K_d(Human)=36.63×10^{-6} M. CD results show that, copolymer interaction with proteins affects the secondary structure of both proteins. Fluorescence emission intensity of complex was decreased by increasing copolymer concentration.

Keywords: Copolymer, Insulin, Binding Affinity, Critical Aggregation Concentration (C.A.C), Nanoparticles.
