Abstract No.135

Analysis of the Conformational Strain of an Unfavorable Residue in the Firefly Luciferase by Site-Directed Mutagenesis

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Firefly luciferase, located in specialized peroxisomes, is the enzyme which catalyses the oxidation of luciferin into oxyluciferin and produces visible light (λmax 557 nm). Residue Thr346 in the 341–348 loop is the only firefly luciferase residue located in a disallowed region of the Ramachandran plot. Energetically unfavourable conformations are rare in proteins and are usually in regions of the structures involved in function. Therefore, to better understand the importance of the conformational strain of Thr346 on luciferase structure and function, we have replaced it by Val and Pro. The changes in protein stability and the effects on the Kinetic properties have been determined.Site-directed mutagenesis was performed for the wild type luciferase gene in the pET-16b vector using the quick-change PCR. The mutant and native luciferases were expressed in E. coli and purified by Ni Sepharose affinity chromatography. Then, the kinetic parametersof purified proteins were determined for luciferases using the luminometer.The results showed that kinetic parameters of mutant enzymes altered by this manipulation. The native form and all mutants exhibit a similar spectrum with only a peak at 557 nm and a clear improvement in thermostability was observed for T346V mutant luciferase compared with the native P. pyralis.Structure engineering of P. pyralis luciferase at position346 has indicated that mutagenesis at this position can change some properties of firefly luciferase and improve the kinetic thermostability of enzyme at 30 °C by T346V mutant. The Km values of mutant luciferases for ATP were increased, indicating lower affinity of the substrates for the mutant enzymes.

Keywords: Firefly Luciferase, Thermostability, P. pyralis, Protein Engineering.

Abstract No.136

Thermodynamic Stability and Structural Studies of Luciferase in Native and Disulfide-bridge Mutant Form

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Luciferase enzyme catalyzes oxidative decarboxylation of luciferin in the presence of Mg^{2+}, O^2 and ATP which leads to generation of light during a multi step reaction. Quantum yield of this reaction is the highest among known bioluminescence systems compared with the amounts of utilized luciferin. Firefly luciferase is rather unstable and commonly loses its activity in room temperature which is because of structural alterations forced by appearance of inner hydrophobic areas of protein. This phenomenon impacts the analytical assay procedure of luciferase. Consequently structural and functional stability of this enzyme is critical for its application. There are different approaches to achieve the increasing stability of this enzyme such as design of covalent bonds like disulfide bonds in mutation procedure.In this study, we investigate disulfide-bridge mutant form of luciferase and compared with the native form. Mutant form of the enzyme is characterized by A296C-A326C.Thermodynamics and biophysical studies were done by UV-vis, fluorescence and circular dichroism spectroscopies and luminometer. Luminescence studies by differential scanning calorimetry were also performed. Results shown that the mutant form with disulfide-bridge is more enzymatic activity, stable and thermal reversible than native. As a conclusion, increasing the activity of the disulfide bridge mutant form is relevant to expanding of active site that is confirmed by bioinformatics study.

Keywords: Luciferase, Disulfide Bridge Mutation, Luminometer, Differential Scanning Calorimetry, Reversibility, Stability.

Abstract No.137

Prediction of Gamma-B Crystallin Stability Upon Point Mutation at Phe56

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Crystallin proteins (alpha, beta and gamma) comprise 90% of eye lens proteins. While alpha-crystallin exhibits chaperone activity and is

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