Abstract No.135

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

Maryam Moradi [1], Saman Hosseinkhani [1], Rahman Emamzadeh [2]*

1. Department of Biochemistry, Faculty of Biological Science, Tarbiat Modares University, Tehran, IR
2. Department of Biology, Faculty of Science, University of Isfahan, Isfahan, IR

(E-mail: m.moradi275@gmail.com)

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

Mina Naderi [1], Saman Hosseinkhani [2], Ali Akbar Moosavi Movahedi [1], Mosa Bohlooli [1]*

1. Institute of Biochemistery & Biophysics, University of Tehran, IR
2. Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, IR

(E-mail: minanaderi@hotmail.com)

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

Sara Zafaranchi [1], Reza Yousefi [1], Sadaf Saba [1], Mohadeseh Kashani [1], Abolfazl Barzegar [2]*

1. Protein Chemistry Laboratory (PCL), College of Sciences, Shiraz University, Shiraz, IR
2. Research Institute for Fundamental Sciences (RIFS), University of Tabriz, Tabriz, IR

(E-mail: sara_zaafaranchi@yahoo.com)

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