

On the other hand, Sucrose and Trehalose are natural osmolytes stored in cells of organisms in order to adapt them to environmental stresses. In vitro, osmolytes decrease protein instability and force partially unfolded structures to refold. In this study, we have done Molecular dynamics simulations of firefly Luciferase in pure water and sucrose and trehalose to investigate the molecular basis of the stabilizing effect. Our results show that sucrose and trehalose mainly interact with protein main chain and water preferentially interacts with protein side chain. Also as sucrose and trehalose H-bonds with solvent decrease, their H-bond with main chain and side chain of protein increase. In addition comparison of pure water with these osmolyte solutions shows some difference in root mean square fluctuation and secondary structure of protein. As well the simulation data indicates that presence of osmolytes influence water structure and make it more ordered. Thus, these findings suggest that osmolytes probably by indirect interactions prevent protein thermal unfolding.

Keywords: Luciferase Enzyme, Co solvents, Molecular Dynamics Simulation, Protein Structural Stability.

Abstract No.43

Molecular Modeling and in Vitro Studies on Proposed Inhibitor of Bacterial Alpha-amylase

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Normal flora of the oral cavity have a role in the formation of dental caries, which occur by fermentation of carbohydrates. As so, bacterial alpha-amylase enzymes, such as Streptococcus mutans and Streptococcus sanguinis enzymes can be considered as suitable targets to prevent this event. In this study, for each bacterial enzyme, 11 models were built by Moe software. After quality evaluation, best models were selected for further studies. Potential inhibitor compounds were docked into the structures with the use of Autodock Vina. In the experimental part, the effect of gibberellic acid was studied on Bacillus amyloliquefaciens alpha-amylase, as a model of bacterial enzyme, with the use of Bernfeld method. Blast and Clustal W2 programs showed more than 70% similarity between bacterial and human salivary alpha-amylase sequences. Comparison of metal ion binding sites and active

sites showed acceptable similarity between the target and template structures. The results of docking showed a high affinity between gibberellic acid and bacterial enzyme. In experimental study, gibberellic acid was able to inhibit enzyme activity up to 15% at concentration of 20 μM. Gibberellic acid could be further studied as to its inhibitory potential on other bacterial amylases.

Keywords: Alpha- Amylase, Molecular Modeling, Inhibitor.

Abstract No.44

Investigation of His-His interaction on the TMGS stability

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Methylglyoxal synthase is a homohexameric protein that catalyzes an elimination reaction that converts dihydroxyacetone phosphate (DHAP) to form orthophosphate and methylglyoxal, which very applicable in industry. For this reason, MGS study and improved its stability is important. Recently, a gene encoding MGS from Thermus sp. GH5 (TMGS) was cloned, overexpressed and its structure has been studied by X-ray crystallography. These studies showed that TMGS gene was composed of 399 bp which encoded a polypeptide of 132 amino acids with a molecular mass of 14.3 kDa. The crystal structure of TMGS revealed that His23, Arg22 and Phe19 are in closed distance and forming a loop. On the other hand our recent study has shown that His-His interaction may be important in protein stability. To further explore this phenomenon, in this survey two modified enzymes were produced with site directed mutagenesis (SDM), one of them, one histidine and another, two histidine inserted between Arg22 and His 23. After sequencing, expression and purification, thermal stability measurements performed and the data exhibited that the thermostabilities of both mutants improved compared native TMGS. These mutagenesis data support this hypothesis that the interactions between like-charged amino acid residues can improve protein stability.

Keywords: Methylglyoxal Synthase, Thermophile, His-His Interaction, Stability.