INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Effect of Confinement on Protein Conformation in Presence of Osmolytes Urea and Trimethylamine-N-Oxide: Replica Exchange Molecular Dynamics Simulation Study

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SHORT ABSTRACT

The mechanism by which TMAO provides counteraction against the action of urea and stabilizes protein has been investigated. We focus on the effects of urea and TMAO on the folding/unfolding equilibrium of protein and put an effort to uncover the molecular basis of denaturation and counteraction mechanisms. In Chapter 1, we present a detailed literature review of the mechanism of action of the osmolytes, their effects on the protein. The reason of choosing REMD (Replica Exchange Molecular Dynamics) simulation technique over the classical MD technique is also explained. In Chapters, we have performed a comparative study utilizing Kast and Osmotic models of TMAO to examine the extent of protein protecting ability of the two models. We have tried to explore the underlying mechanism by which urea and TMAO exert their effects on the protein β-hairpin. Urea causes denaturation of the protein through breaking of the terminal intra-protein hydrogen bonds. Kast model of TMAO, in conventional 2:1 ratio of urea:TMAO provides counteraction against denaturing effect of urea. Osmotic model provides maximum counteraction when used in 1:2 ratio, but fails to provide sufficient conformational stability to the protein in conventional 2:1 ratio of urea and TMAO. These findings lead us to carry out further studies with Kast model of TMAO. In Chapter 3, we have emphasized on the counteraction behavior of TMAO against action of urea without using any confinement. Using REMD simulation technique we have studied urea-induced denaturation and TMAO-induced counteraction of the protein Trp cage. We have particularly emphasized on three factors that persuade stability on the protein Trp cage: (i) salt bridge between residues Asp9 and Arg16, (ii) the buried hydrophobic core surrounding the indole plane of residue Trp6 and (iii) orientational preference of different aromatic planes constituting the protein with respect to the indole plane of residue Trp6. Large number of protein-urea hydrogen bonds indicate direct interaction of urea with the protein that result in unfolding of the protein. Small number of protein-TMAO hydrogen bonds provides insufficient support for direct interaction mechanism of TMAO with the protein.

In Chapter 4, we have examined the effects of non polar confinement on the folding/unfolding equilibrium of the protein Trp cage by means of REMD simulation technique. We have used a near spherical non polar fullerene ball consisting of 2940 carbon atoms in order to mimic the role of chaperonin unit in protein folding. Free energy landscapes (FELs) are calculated as a function of temperature and RMSD (root mean square deviation) that depict conformational space visited by the protein. Analysis of density profiles of water for pure water system reveals that there is dewetting of the hydrophobic surface of the fullerene ball. In binary urea solution all the residues of the protein show high probability to remain near the surface of the fullerene ball. Under the effect of confinement urea molecules get more access to solvate the protein that cause denaturation. In Chapter 5 we have studied the effects of polar confinement on the folding/unfolding equilibrium of the protein Trp cage. We have used a spherical polar confinement consisting of 2940 carbon atoms with randomly distributed charges on the different carbon atoms of the fullerene like ball. Comparison of FELs and other properties like radius of gyration, RMSF, DSSP indicate that in polar confinement the folding propensity of the protein becomes lower than that in non polar confinement. Urea induced denaturation is found to be more effective in presence of polar confinement. Equivalent folding propensity of the protein in pure water and mixed ternary solution indicates that TMAO counteracts urea's action effectively in presence of polar confinement. Analysis of density profiles of water ensures an enriched layer of water that wets the polar surface of the confinement. The protein residues in all the solutions show small tendency to occupy peripheral region of the confinement. Calculation of intra- and intermolecular hydrogen bonds are also employed to analyze the mechanisms of urea and TMAO inside the polar confinement. In Chapter 6, we have discussed the results of each of the work and tried to bring our view on the counteraction mechanism of TMAO and the effect of confinement on the protein folding/unfolding equilibrium. A comparative view of the effect of polar/non polar confinement on protein conformation is also elucidated.