Studies on the interaction of some biologically relevant small molecules with proteins: Insights on stabilization, displacement and distribution of ligands and associated structural aspects

Biological systems are very complex and emerge out of various types of interactions therein. Out of that, binding interaction of small molecules with proteins plays some important roles to steer the biological processes in every living system. Most of the cases, the binding is noncovalent and reversible in nature, and affinity of binding controls stabilization, displacement and distribution of small molecule in the protein medium. The protein structures can be quite flexible to bind the small molecules, and in the majority of cases, the natures of binding produce some significant effects on the overall activity profile of the molecule as well as the protein. In the thesis, I have explored the pattern of structural changes of the biomacromolecule, i.e., proteins as a consequence of the binding of small molecules with it from the perspectives of distribution of ligands, stabilization of hydrophobic drugs and competitive binding of the drugs.

The interaction of lysozyme, namely, human lysozyme (HLZ) and chicken egg white lysozyme (CEWLZ) with azo food colorant, tartrazine (TZ) has been investigated in physiological pH condition. The binding interaction of TZ is mostly electrostatic in nature in the hydrophobic pocket of lysozyme. Strong binding of TZ altered the secondary structure more in case of HLZ than that of CEWLZ. Salt-induced changes in the binding strengths and consequent refolding of protein structure indicate that the thermodynamic properties are better compensated in case of CEWLZ-TZ than HLZ-TZ complex. The results provide important information about the binding pattern of lysozyme with an exogenous molecule and salt mediated protein purification. The efficiency of hydrophobic drugs is quite dependent upon its stabilization, and in that case, the role of carrier protein always needs to be understood very well. I have found that solubility and stability of the hydrophobic drug, curcumin are significantly enhanced in the presence of two variety of β-sheet-rich silk proteins, where non-mulberry silk provides better stability of curcumin than mulberry silk having different location of binding sites. In the presence of cocktail of drugs, the distribution of free drugs depends upon the nature of its displacement from protein pocket. In the case of drug-drug-competition (methotrexate, folic acid) in the pocket of transporter protein HSA, it has been found that the binding parameters are largely altered from single drug binding by modifying protein secondary structure and in fact, restoration the structure from a more perturbed to a lesser perturbed state. The combination of drug-drug-competition and protein structural change is considered to be the responsible factors in controlling the distribution of bound and unbound fraction of drugs. Casein is regarded as one of the main protein sources in the diet as all as it carries numerous small molecules including fatty acids. It is found that unsaturated fatty acids bind strongly with casein than that of saturated fatty acids by imparting hydrophobic nature of interaction in casein unit. With alteration of hydrophobic nature of interaction, microenvironment and size are considerably change to show a little-ordered structure (α-helix) from the disordered one. The rigid and hydrophobic unsaturated fatty bound casein can be a suitable system for oral drug delivery system for hydrophobic drugs. I have found that with addition of casein, the size of lipid liposome decreases and along with increases the rigidity in the microenvironment. That smaller and rigid casein mixed liposome provide greater stability of hydrophobic molecule curcumin and β-carotene than the pure systems.

Keywords: Lysozyme, small molecule, enthalpy-entropy compensation (EEC), silk protein, secondary structural change, refolding, drug-drug competition (DDC), casein, hydrophobic environment, microenvironment, rigidity, drug delivery system (DDS), and casein mixed liposome.