Studies on Site-Specific Interaction of Fluorophores and Drug Molecules with Serum Albumins and Their Possible Applications

Abstract: The principal focus of the thesis is to explore site-specific interaction of different ligands (fluorophores and drug molecules) with two homologous serum proteins, human serum albumin (HSA) and bovine serum albumin (BSA) and to suggest some possible applications exploiting such interactions. Here binding of serum proteins (HSA and BSA) with a water-soluble azo food colorant, carmoisine has been investigated using optical spectroscopy and molecular docking studies. Site-specific binding of the dye in the subdomain IIA of HSA and in subdomains IIA and IB of BSA modifies the secondary structure of proteins and this is accompanied by alterations of microenvironment of tryptophan(s). The interaction is found to be pH-insensitive which can have relevance to the toxicological profiles of the dye, and the observed ionic strength dependence of binding can be advantageous in protein purification. A comprehensive understanding of the effect of subdomain IIA pocket-specific interaction on acid-base equilibrium of housed guests has been presented. Electrostatic interaction is found to play a dominant role in the binding of the guests, especially charge carrying molecules, in the said cavity. The pK_a of subdomain IIA binder basic drugs decreases due to unfavorable interaction of cationic form with the positively charged amino acid side-chains in the cavity. On the other hand prototropic equilibrium of acidic drugs is shifted towards the anionic form due to favored binding of the deprotonated species presumably *via* electrostatic interaction with anion receptors. Acidity-shifting efficacy of albumins is introduced for the first time using pK_a-shifting index (α), a unique parameter for given prototropic-drug-host pair to assess drug bioavailability. Acidic drug warfarin and basic fuberidazole, showing high α-value, should be efficient in drug-SA cocktail, and drugs with low α are not expected to be very useful. Shifting of pK_a of protein-encapsulated drugs stems the possibility of albumin-based delivery systems for extracting the therapeutically active species. A site-specific approach, particularly in reference to the subdomain IIA, reveals how the receptor property of serum protein pocket could be important in determining the strength of interaction with exogenous ligands. The individual responses of the serum protein to the perturbation caused by coumarin dyes (coumarin 343-anion and coumarin 314) are found to be way different. Although the major drug binding site (subdomain IIA) of both the proteins has anion receptor property (prefers anionic ligands like deprotonated coumarin 343) but interestingly HSA appears to adjust to some favorable conformation when

required, like a mechanical slide-wrench, to accommodate neutral ligands like coumarin 314 in the pocket as well. On the other hand, due to less flexible solution structure, BSA is not very responsive to ligand perturbation, as it happens with fixed mechanical spanners, and hence its interaction with neutral fluorophore, coumarin 314, is very weak. Site-specific anchoring of a π-conjugated organic molecule in low conducting serum albumins by non-covalent interaction leads to a marked enhancement in electron transport through the protein. Due to binding with serum albumins the photophysical properties of the fluorophore is greatly modified which has been utilized in characterization of microenvironment of the binding cavity in subdomain IB. Hosting foreign substances (e.g., conjugated polyenes) lowers the energy barrier for the non-adiabatic electron transport across the thin layer of albumin which can be exploited in the designing of albumin-based electronic devices, such as biosensors and biocompatible electronic charge-carrying elements.

Keywords: serum albumins, host-guest interaction, site-specific binding, subdomain IIA, anion receptor, electrostatic interaction, thermodynamics, prototropic equilibrium, pK_a-shifting index, albumin-based delivery system, drug bioavailability, conformational adaptability, molecular docking, optical spectroscopy, subdomain IB, electron transport.