Human Serum Transferrin: Role of Ionic Interactions with the Transferrin Receptor in Binding and Iron Release

Iron is an extremely important nutrient, required for oxygen transport by red blood cells, ATP synthesis in mitochondria, and DNA synthesis. However, iron atoms are toxic and insoluble in the aqueous environment of the blood. Due to this paradox, the bilobal glycoprotein human transferrin (hTF) is required to safely and efficiently transport iron from the diet throughout the body. Cellular iron uptake is mediated by a membrane-bound receptor (TFR), which is capable of distinguishing between iron-bound and iron-free hTF. Despite extensive research, questions remain regarding the precise interactions between the TFR and hTF which control this specificity. *In silico* simulations have been used to model the structure of the complex. More recently, an x-ray crystallographic structure of the hTF/TFR complex has been determined by our laboratory. Significant differences in particular ionic interactions have been identified in comparison to the *in silico* model attributed to structural changes in the TFR induced by binding of hTF.

Site-directed mutagenesis of newly identified putative binding residues and recombinant expression and purification of mutant constructs allows analysis of the specific role of individual amino acids in the binding interaction. We have measured binding affinity between hTF and the TFR using isothermal titration calorimetry (ITC). Rate constants for iron release have been determined for these same mutants. Due to the intrinsic fluorescence properties of hTF, iron release kinetics can be measured using a stopped-flow spectrofluorimeter. This technique which is sensitive, rapid, and has an excellent signal to noise ratio allows the capture of all kinetic events.

I will highlight the contribution of individual ionic interactions in the interaction of hTF with the TFR. This work illuminates the limitations of modeling in such a complex system.