

Studies to understand Ti speciation and transport in the human body.

Ti(IV)-containing implants are known to release the metal in the blood. This release increases the Ti concentration nearly 50 times greater (0.25 μM) than people with no such implants. Citrate, present in high μM amounts in blood and synovial fluid, is one of the key species that contributes to the solubilization and chelation of Ti(IV). To study the speciation, we need to consider one important factor that is the pH. The stability of all metal complexes depends on pH. Many Ti(IV) complexes at neutral pH are extremely hydrolysis prone and precipitate as TiO_2 . However, in human blood, the protein human serum transferrin regulates Ti(IV) speciation and protects the metal from hydrolysis. By understanding the aqueous speciation of the Ti(IV) serum transferrin (sTf) complex we will identify how the metal is transported and what is its biological fate. To gain insight into sTf coordination of Ti(IV) coordination we are interested in studying Ti(IV) interaction with small molecule mimics of the protein binding site. The mimics include moieties that represent the two tyrosines and the histidine of the metal binding site. Deferasirox is a tridentate ligand that includes two phenol rings and a triazole ring. This ligand is able to saturate Ti(IV) coordination by binding in a 1:2 metal:ligand stoichiometry. X-ray crystallography with a deferasirox analogue, 3,5-bis(2-hydroxyphenyl)-1,2,4-triazole (BHPT), reveals that the deferasirox molecules coordinate in a meridional fashion. Solution-state structures do not necessarily compare with solid-state structures. We used a suite of spectroscopic techniques to determine the aqueous speciation of the Ti(IV) deferasirox interaction. We identified that between the pH 4 to 8, the metal and ligand form exclusively 1:2 metal:ligand complexes at micromolar concentrations. We performed potentiometric studies to determine the pKa values of the carboxylic groups of deferasirox when the ligand coordinates the metal at the 1:2 ratio ($\text{pK}_{\text{a}1} = 5.7 \pm 0.2$ and the $\text{pK}_{\text{a}2} = 5.9 \pm 0.3$). These pKa values indicate that the neutral complex $\text{Ti}(\text{def})_2$ dominates at the pH range of 4 to 5.5, $\text{Ti}(\text{def})_2^{1-}$ (loss of one carboxylate proton) is present at pH range of 5.5 to 5.9 and $\text{Ti}(\text{def})_2^{2-}$ (loss of one carboxylate proton) exists at pH range pH of 6.0 to 7.0. In addition to understand Ti(IV) blood speciation, we will explore the transport mechanism of the metal into cells via the sTf endocytotic route. To study this transport, we will create a fluorescent version of citrate, a molecule that remains bound to Ti(IV) when it becomes sTf bound. The synthesis and purification of this fluorescent citrate is described. This probe will help us elucidate the intracellular localization of Ti(IV).