Effect of Chloride on the Rates of Iron Removal from Monoferric Transferrins by Pyrophosphate

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Transferrin is a glycoprotein in the serum of vertebrates and some invertebrates. Iron removal kinetics by various ligands have been studied, and pyrophosphate is one of the more effective agents for iron release. Pyrophosphate solutions at pH 7.4 can be prepared in two ways. One can add a base such as NaOH to pyrophosphoric acid ($PP_i(H)$), or one can add a strong acid such as HCl to a solution of tetrasodium pyrophophate ($PP_i(Na)$). In the latter case, the solution will contain an equimolar concentration of the chloride anion. Since numerous studies have shown that the presence of inorganic anions can affect the rates of iron release, we have conducted kinetic studies with both forms of pyrophosphate to determine whether the method of preparation has a significant impact on the kinetic results.

The rates of iron release from both C-terminal and N-terminal monoferric transferrin were measured by visible spectrophotometry for a series of pyrophosphate concentrations. Iron release from both monoferric transferrins shows a complex dependence on the ligand concentration. The reaction appears to follow saturation kinetics at low ligand concentrations and first-order kinetics at high ligand concentrations. For C-terminal monoferric transferrin, the rate of iron release was slightly faster for $PP_i(H)$ vs $PP_i(Na)$, but the difference was relatively small. For N-terminal monoferric transferrin, the rates of iron release by $PP_i(H)$ were significantly faster than with $PP_i(Na)$. More specifically, there was a 50% reduction in the rate of iron release via the first-order component by $PP_i(Na)$.

We previously reported that the rate of iron release by pyrophosphate from the C-terminal site was faster than the rate of release from the N-terminal site. In now appears that this result was an artifact resulting from the presence of chloride in the $PP_i(Na)$ solution. When a chloride-free $PP_i(H)$ solution is used, there is little difference in the rates of iron release from the two transferrin binding sites.