Transferrin: The Role of Conformational Change in Iron Removal by Chelators. Lactoferrin: The Role of Conformational Changes in Its Iron Binding and Release.

Raymond, K. N. *et al. J. Am. Chem. Soc.* **1993**, *115*, 6758-6764.

Raymond, K. N. *et al. J. Am. Chem. Soc.* **1993**, *115*, 6765-6768.

UV-Topic: UV Kinetics, Derivative Spectroscopy

Chem Topic: Metal binding in enzymes

Serum transferrin is the iron transport agent and iron buffer for mammals. Transferring is a bilobal protein (MW 78000) formed by fusion of two ancestral proteins. Each lobe contains a metal-binding site with high affinity to high spin Fe(III). Carbonate binding to transferrin is a prerequisite to iron binding and release ("synergistic anion", "mediator anion"). It is now becoming clear that these anions are required to promote conformational change.

The authors explore the differences in the behavior of prototypical ligands for iron removal via UV/Vis kinetics. The kinetics of iron removal from diferric transferrin by the catecholeate and hydroxamate chelators, 3,4-LICAMS and desferrioxamine B, were investigated in the presence of the mediator PPi (tetrasodium pyrophosphate). Similar investigations with monoferric transferrin.

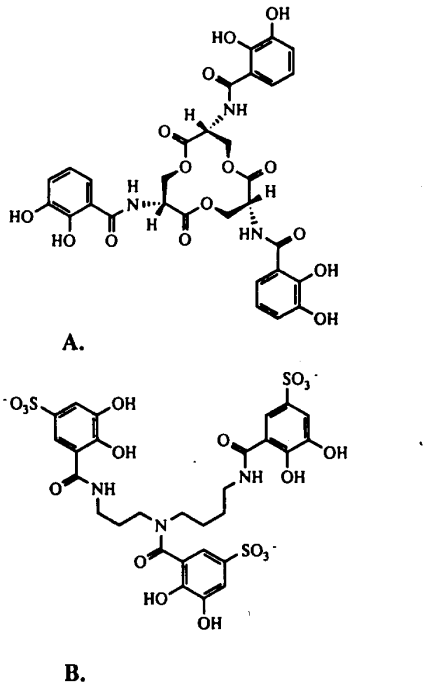

Figure 1. Structural formulas of (A) enterobactin and (B) 3,4-LICAMS (1,5,10-N,N′,N″-tris(5-sulfo-2,3-dihydroxybenzoyl)triazadecane).

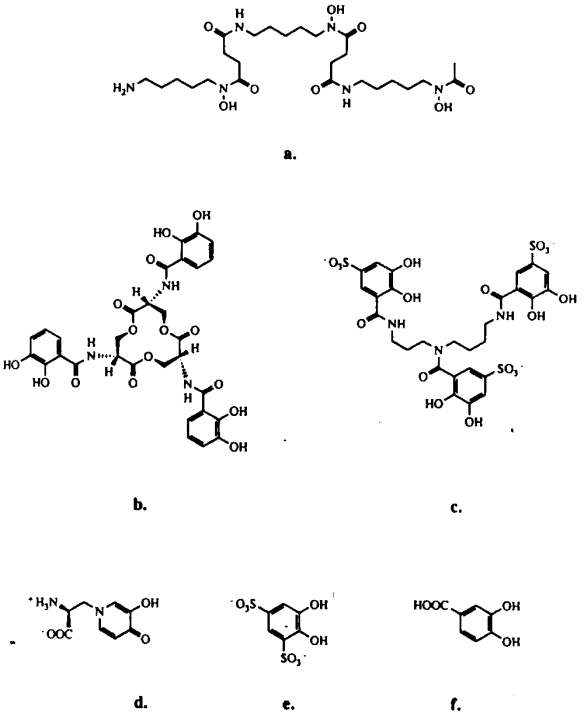

Figure 1. Structures of ligands discussed in this paper: (a) desferrioxamine B; (b) enterobactin; (c) 3,4-LICAMS; (d) L-mimosine; (e) Tiron; (f) 3,4-dihydroxybenzoic acid (DHBA).

Kinetics of Iron Removal from Transferrin with L-Mimosine

Addition of mimosine to diferric transferrin in 0.050 M HEPES buffer results in shift from 466 nm to 450 nm (complex with three mimosines). Kinetics monitored at 466 nm depending on ligand concentration and under pseudo-first order conditions (more ligand than iron). Note the saturation behavior.

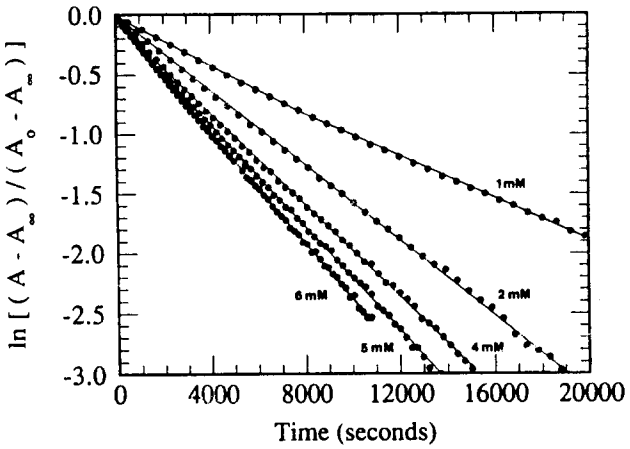

Figure 2. Iron removal from 0.100 mM diferric transferrin by various concentrations of L-mimosine (0.050 M HEPES buffer, pH 7.4, 25 °C, μ = 0.026).

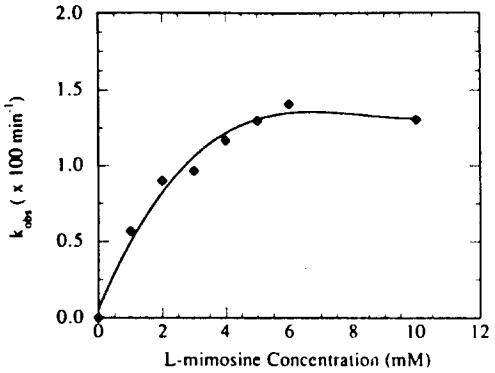

Figure 3. Plot of observed rate constants as a function of increasing concentrations of L-mimosine as determined by linear-least-squares analyses for iron removal from 0.100 mM diferric transferrin (0.050 M HEPES buffer, pH 7.4, 25 °C, μ = 0.026).

Lactoferrin is an iron-binding protein in the same family as serum transferrin which occurs in traces only in mammals. Its most important function consists in keeping iron concentrations below levels required for bacterial growth. In this paper, the authors contrast the 260-fold greater stability of lactoferrin relative to transferrin and they address the origin (higher affinity or slower release).

Kinetics of Iron Removal from Lactoferrin with 3,4-LICAMS

At 37°C, much the same as above. Chelator addition shifts to higher wavelength and increases intensity as the complex is formed. Kinetics were followed for various chelator concentrations.

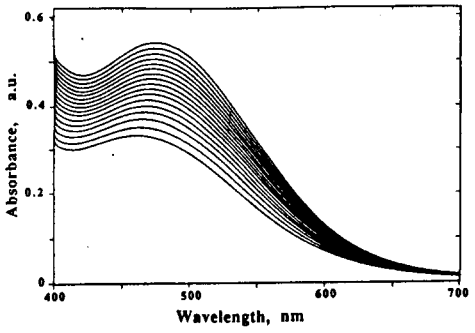

Figure 2. Spectral changes accompanying iron removal from transferrin. The bottom curve represents unreacted transferrin, and the top curve, the final product ferric 3,4-LICAMS. Conditions: 0.1 M Tris-HCl, pH 7.4, 37 °C; [Tf] = 0.062 mM, [LICAMS] = 12.3 mM, ambient CO_2. Each spectrum was taken at successive 2-min intervals after the start of reaction.

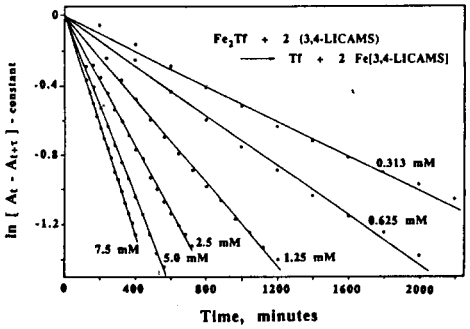

Figure 3. Guggenheim plots for iron removal from diferric transferrin (0.0625 mM) by various concentrations of 3,4-LICAMS at 0.1 M Tris-HCl, pH 7.4, ambient CO_2, and 37 °C.

The new things here is this: How do you determine that there are only two absorbing species (that is that you are following a clean reaction) in the absence of isosbestic points? One uses the derivative spectrum which does have such quasi-isosbestic points and which can be similarly interpreted. Fig 6 and 7 show these isosbestic points.

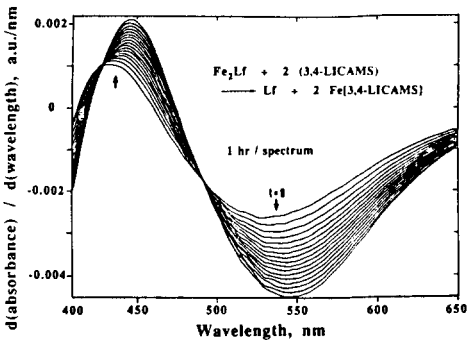

Figure 6. Derivative spectra obtained during iron removal from diferric lactoferrin by 3,4-LICAMS. Derivative spectral changes increase with time; each spectrum was taken at successive 1-h intervals after the start of the reaction.

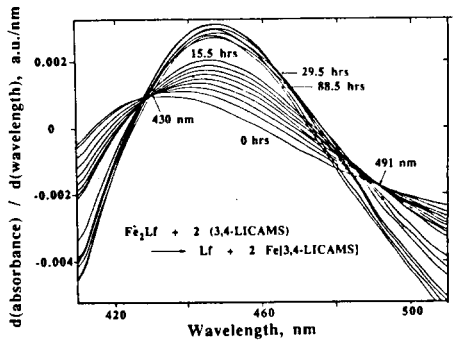

Figure 7. Derivative spectra at very early and long times for iron removal from diferric lactoferrin by 3,4-LICAMS.