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Kinetics of Aqueous Iron(III) Complexation by Desferrioxamine B

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SUMMARY

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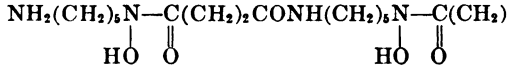
Light absorbance stopped-flow rate measurements have been made of the formation of a complex between iron(III) and desferrioxamine B in 0.1 M ionic strength, acidic, aqueous solution at and below 36°. The over-all second-order rate constant for the reaction iron(III) + desferrioxamine B → 1:1 complex is $k_2 = 2.5 \times 10^3\ M^{-1}\ sec^{-1}$ at pH 3.92 and 36°. This is of the same order of magnitude as previously reported rate constants for aqueous iron(III) complexation by various ligands. The over-all second-order rate constant for the same reaction in blood plasma for pH values ranging from 4.1 to 7.1 at 36° is the same as in aqueous solution to within experimental error. Thus, at physiological pH and temperatures the actual complexation reaction is sufficiently rapid that one may expect this ligand attached to a polymeric substrate to remove excess iron from blood more rapidly than the blood can be circulated in an extracorporeal cleansing device.

INTRODUCTION

Supplementary iron is one of the most prevalent causes of accidental poisoning of children in the United States. One of the methods of treatment is exchange transfusion. Losses of proteins, lipids, and other valuable substances from the body through this process are serious (1). Metal chelation, with the consequent enhanced urinary excretion, appears to be a more promising form of treatment. One such chelate is desferrioxamine B,



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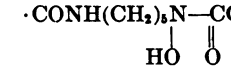

discovered (2) in Switzerland in the form of the methanesulfonate salt.

A compilation of complex ion stability constants (3) reveals a peculiar selectivity of desferrioxamine B for iron(III) compared to two other metal ion-chelating agents which have been used therapeutically: ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid. For instance, for Ca^{2+} the stability constants are $10^{2.6}$, 10^{11}, and 10^{10} with these three chelating agents, whereas for Fe^{3+} they are $10^{30.6}$, 10^{25}, and 10^{29}, respectively. Although desferrioxamine B complexation of iron(III) has been studied as an equilibrium problem (4–6), kinetics of complex ion formation has evidently not been reported. The kinetics and mechanism of complexation by a hexadentate ligand do not, in general, lend themselves to facile investigation by relaxation techniques such

as the temperature-jump method (7). The present unavailability of stability constants for the several partially coordinated intermediate ions, as well as the overwhelmingly large over-all stability constant for the fully coordinated complex ion, completely preclude such a relaxation study. However, an investigation of the kinetics of the over-all reaction leading to the fully coordinated 1:1 iron(III)–desferrioxamine B complex ion by the stopped-flow technique (8) does have some practical interest.

Specifically, the iron(III)–desferrioxamine B complex is toxic, producing renal shutdown, hypotension, and shock (1). Thus an effort is being made to place desferrioxamine B on polymeric substrates. Ramirez and Andrade (9) have coupled the desferrioxamine B to polyacrolein and have found that the compound will remove iron(III) from aqueous solution. This will, in principle, permit removal of excess iron from blood circulated outside the body. For this reason it is important to know the time required for iron(III)–desferrioxamine B complex formation as well as the pH dependence of the kinetic process.

Two further features of this complexation kinetics study should be noted. The strong absorbance of the iron(III)–desferrioxamine B complex at 430 nm makes the reaction rate particularly easy to follow in dilute solutions with a stopped-flow apparatus. Also, aqueous iron(II) of the ubiquitous ferrous sulfate dietary iron supplement is so readily oxidized to iron(III) that only the complexation of the latter iron by desferrioxamine B can have medicinal significance.

EXPERIMENTAL PROCEDURE

Materials. Desferrioxamine B methanesulfonate was obtained from Ciba Corporation and used without further purification; m.p. 140–142°. The iron used was anhydrous ferric chloride supplied by Matheson, Coleman, and Bell. Buffers were made from reagent grade potassium acid phthalate and potassium chloride. All water used in the experiments was distilled and then demineralized.

The human blood plasma used in these experiments was supplied by Cutter Laboratories. It was used without further modification. To facilitate the dissolution of the ferric chloride in the plasma, the pH of the plasma was reduced. The pH was then adjusted using sodium hydroxide.

Stopped-flow apparatus. All kinetic experiments were carried out on a Durrum-Gibson model D-110 stopped-flow apparatus. In the stopped-flow technique (8) the two reactant solutions are forced into a chamber and rapidly mixed. Liquid flow is stopped so that the mixed solution comes to rest within 2 msec of mixing. The reaction is monitored spectrophotometrically with a logarithmic amplifier so that an optical density vs. time display appears on the oscilloscope screen. A typical oscillograph is shown in Fig. 1.

Kinetic experiments. Hereafter we shall denote the desferrioxamine B cation by H_4DFO^+. It is the complex $Fe(HDFO)^+$ that absorbs light strongly (6) near the 430-nm wavelength at which all stopped-flow measurements were made. In all aqueous experiments the initial concentrations of iron(III) and desferrioxamine B were set equal to one another. These concentrations ranged from 86 to 410 μM. Higher concentrations produced unacceptably high optical densities.

All aqueous solutions were buffered: those in the 1.5–2.0 pH range by potassium chloride and hydrochloric acid, and those in the 2.1–3.9 pH range by potassium acid phthalate and hydrochloric acid. Kinetic experiments above pH 3.9 in aqueous solution were not completed because of precipitation in the buffered iron(III) solutions.

Ionic strength was adjusted with KCl to 0.1 M, and in most of the kinetic experiments the temperature was 36° ± 0.05°.

The kinetic experiments using human blood plasma as the solvent were similar to those in aqueous solution. Concentrations of iron(III) chloride and desferrioxamine B were set equal to one another at 12 μM. No buffers were added. The pH of the plasma solutions ranged from 4 to 7.

Attempts at pseudo-first-order rate studies were unsuccessful. In such experiments three different slopes on individual oscillograms were observed that possibly could be explained in terms of the stepwise formation of

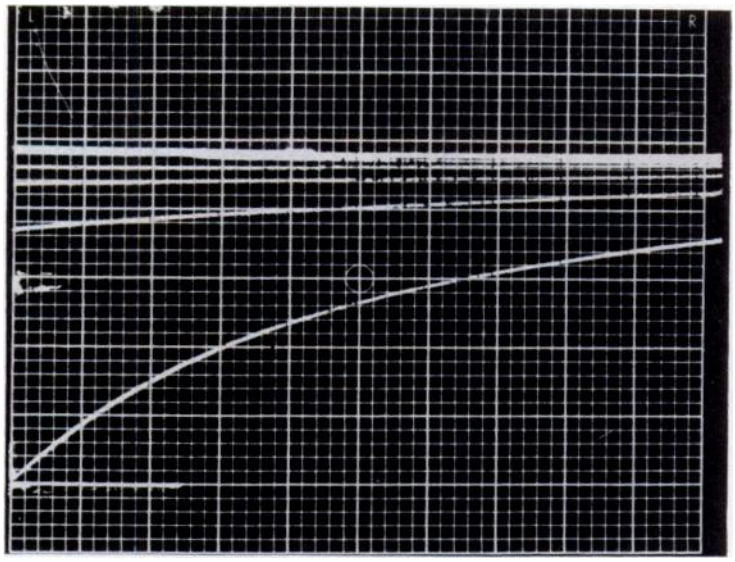

FIG. 1. *Oscilloscope trace for reaction of iron(III) with desferrioxamine B in 0.1 M ionic strength aqueous solution at pH 2.10 and 36°*

The initial concentration of each reactant is $c_0 = 259\ \mu M$. The vertical scale is optical density units: each major division corresponds to 2 V or 0.2 optical density unit. The bottom horizontal trace corresponds to 100% transmission at 430 nm. The horizontal trace five major divisions above this trace corresponds to an optical density of unity. The horizontal time scale is 5.0 sec/major division.

intermediates in the over-all 1:1 complex formation process. Because the reproducibility of the curves was marginal and stability constants were not determined for these intermediates, this approach to the kinetic problem was abandoned.

RESULTS

As is evident from a correlation coefficient of 0.999 for a linear plot of data extending over three half-lives, the complexation reaction

$$\text{Iron(III)} + \text{desferrioxamine B} \xrightarrow{k_2} \text{Fe(HDFO)}^+ \qquad (1)$$

is described very accurately by the integrated second-order rate expression

$$\frac{x}{c_0(c_0 - x)} = k_2 t \qquad (2)$$

where c_0 is the initial concentration of iron(III) as well as desferrioxamine B, x is the extent of reaction, and t is the time. Numerical values of the second-order over-all rate constant k_2 for the aqueous experiments are given in Table 1, and those for the human blood plasma experiments are given in Table 2. These rate constants are of the same general magnitude as those previously reported (Table 3) for the formation of mono complexes of iron(III) with unidentate ligands at 25°.

From kinetic measurements made at three different temperatures it was also possible to deduce the following activation parameters for the over-all complexation reaction: $\Delta H\ddagger = 12.5$ kcal/mole, $\Delta S\ddagger = -43.2$ e.u.

DISCUSSION

The interesting pH dependence of the over-all rate constant k_2 depicted in Fig. 2 shows that the reaction is more properly described by

$$\frac{d[\text{Fe(HDFO)}^+]}{dt} = k' \frac{[\text{Fe}^{3+}(\text{aq})][\text{H}_4\text{DFO}^+(\text{aq})]}{[\text{H}^+(\text{aq})]^2} \qquad (3)$$

TABLE 1

Representative kinetic data for reaction of iron(III) with desferrioxamine B in 0.1 M ionic strength aqueous solution

c_0[a]	k_2[b]	pH[c]	Temperature[d]
M	M^{-1} *sec*$^{-1}$		°C
8.63×10^{-5}	5.34×10^{2}	1.55	36
3.45×10^{-4}	4.53×10^{2}		
4.14×10^{-4}	5.14×10^{2}		
1.38×10^{-4}	1.08×10^{3}	2.10	36
2.59×10^{-4}	1.19×10^{3}		
4.14×10^{-4}	1.13×10^{3}		
8.63×10^{-5}	2.13×10^{3}	3.50	36
1.38×10^{-4}	2.23×10^{3}		
4.14×10^{-4}	2.54×10^{3}		
8.63×10^{-5}	2.42×10^{2}	1.65	31
2.59×10^{-4}	2.28×10^{2}		
4.14×10^{-4}	2.35×10^{2}		
8.63×10^{-5}	0.87×10^{2}	1.65	26
2.58×10^{-4}	1.46×10^{2}		
4.14×10^{-4}	1.50×10^{2}		

[a] Initial concentration of iron(III) (or desferrioxamine B) in the mixed solution.

[b] Second-order rate constant for the reaction iron(III) + desferrioxamine B → 1:1 complex, calculated from Eq. 2 of the text.

[c] Glass electrode pH of buffered reactant and product solutions.

[d] All temperatures ±0.05°.

TABLE 2

Rate constants for reaction of iron(III) with desferrioxamine B in human blood plasma at 36°

pH	k_2
	M^{-1} *sec*$^{-1}$
4.06	2.21×10^{3}
4.99	2.32×10^{3}
6.07	2.26×10^{3}
7.12	2.27×10^{3}

than by the simpler rate expression,

$$\frac{d[\mathrm{Fe(HDFO)^+}]}{dt} = k_{obs}[\mathrm{Fe^{3+}(aq)}][\mathrm{H_4DFO^+(aq)}] \quad (4)$$

TABLE 3

Rate constants for formation of 1:1 complexes of iron(III) with some monodentate ligands at 25°

Ligand	Rate constant, k		References
	Fe^{3+} + ligand	$FeOH^{2+}$ + ligand	
	M^{-1} *sec*$^{-1}$	M^{-1} *sec*$^{-1}$	
Cl^-	9.4	1.1×10^{4}	10
SCN^-	1.27×10^{2}	1.0×10^{4}	11
SO_4^{2-}	$\sim 6.37 \times 10^{3}$[a]	3×10^{5}	12, 13
HF	11.4	$\sim 3.1 \times 10^{3}$[b]	14
HN_3	4.0	$\sim 6.8 \times 10^{3}$[b]	15
H_2O	2.8×10^{2}	$\sim 2 \times 10^{4}$	15, 16

[a] Calculated assuming that the acid-independent path is $Fe^{3+} + SO_4^{2} \rightarrow FeSO_4^+$.

[b] Calculated assuming that the acid-independent path is $FeOH^{2+} + HX \rightarrow FeX^{2+} + H_2O$.

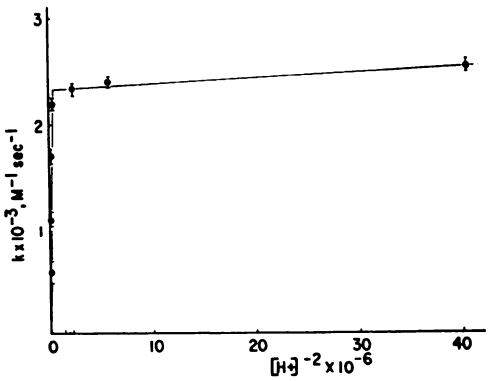

FIG. 2. *Plot of observed second-order rate constant (as in Table 2) of complexation of iron(III) by desferrioxamine B in 0.1 M ionic strength aqueous solution at 36° vs.* $[H^+]^{-2}$

The hydrogen ion concentration, $[H^+]$, was calculated from the measured pH, using $[H^+] = 10^{-pH}/\gamma_{\pm}$ and an activity coefficient $\gamma_{\pm} = 0.76$, valid at 36° and $\Gamma/2 = 0.1$ M. Vertical error bars through the experimental points indicate average errors (not standard deviations). The straight lines are least-squares fits of two groups of data points.

that one would have inferred from the fit to Eq. 2 at a single pH.

The first three acid pK values of desferrioxamine B are reported (4) to be 8.39, 9.03, and 9.70, respectively. Thus essentially all the acid is present as the cation H_4DFO^+ for pH < 3.9. However, the

pK of Fe^{3+} (aq) associated with the equilibrium

$$Fe^{3+}(aq) \rightleftarrows FeOH^{2+}(aq) + H^+(aq) \quad (IA)$$

is ~2.47 at 25° or ~2.28 at 35° in 0.01 M aqueous $NaClO_4$ (17). Thus $FeOH^{2+}$ (aq) supersedes Fe^{3+} (aq) as the predominant iron(III) species in the reactant solutions considered here as the pH in the kinetic measurements is increased from 1.5 to 3.9. This fact, combined with the knowledge that $FeOH^{2+}$ (aq) reacts more rapidly with ligands than does Fe^{3+} (aq) (18) (Table 3), accounts qualitatively for the precipitous increase in k_2 with increasing pH just below pH 3.

A possible reaction mechanism that yields a rate expression identical with Eq. 3 is reaction IA plus the following three additional steps:

$$FeOH^{2}(aq) + H_4DFO^{+}(aq) \rightleftarrows Fe(H_3DFO)^{3+}(aq) + H_2O \quad (IIA)$$

$$Fe(H_3DFO)^{3+}(aq) \rightleftarrows Fe(H_2DFO)^{2+}(aq) + H^+(aq) \quad (IIIA)$$

$$Fe(H_2DFO)^{2+}(aq) \rightarrow Fe(HDFO)^{+}(aq) + H^+(aq) \quad (IVA)$$

with step IVA taken to be rate-determining. The substitution of

$$Fe^{3+}(aq) + H_4DFO^{+}(aq) \rightleftarrows Fe(H_3DFO)^{3+}(aq) + H^+(aq) \quad (IIB)$$

for IA and IIA at lower pH values gives rise to precisely the same rate expression, Eq. 3 above.

The measured $1/[H^+]^2$ dependence of the reaction rate is the only basis for the assumed rate-determining character of step IVA. Presumably, in this step the third hydroxamic acid group is closing on the iron ion to complete the 1:1 complex. One might imagine that this slower reaction step of the bulky ligand is sterically controlled in a manner analogous to that of the reaction of cobalt(II) with β-alanine (19). However, proton loss and closure of a chelate ring are rate-determining in very few systems. As is evident from Table 3, there are pronounced variations in Fe^{3+} substitution rates among ligands of identical charge type, so that the apparent slowness of step IVA could also simply reflect the ligand dependence exhibited by an associative substitution mechanism, in addition to, or in lieu of, steric control.

A comparison of the second-order rate constants found in aqueous solution (Table 1) with those in human blood plasma (Table 2) indicates that there is no difference in the rate of reaction in these two media within experimental error. Thus, unless the stereochemical configuration of desferrioxamine B is significantly changed when attached to a polymeric substrate, the actual iron(III) complexation will not be the rate-determining process in an extracorporeal removal of excess iron from the blood as envisioned by Andrade and Ramirez (9).

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