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Kinetics and Mechanism of the Final Stage of Ferrioxamine B Aquation in Aqueous Acid

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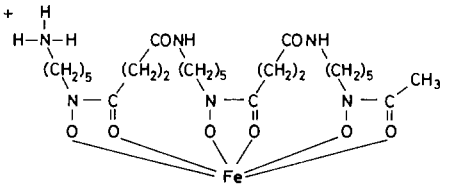
We wish to report the kinetics and mechanism of the last stage of the aquation of ferrioxamine B in acidic solution and its relationship to the corresponding process for a series of synthetic monohydroxamatoiron(III) complexes [1]. Ferrioxamine B is one of a class of linear trihydroxamic acid siderophores whose structure is shown in (I).



(I)

Ferrioxamine B
$Fe(HDFB)^+$

The siderophores are a class of microbially generated compounds whose biological function is to solubilize iron and transport it to the cell where it is then utilized for various processes essential to life [2, 3]. Mechanisms for iron release from its siderophore complex are then of some significance. Ferrioxamine B is of particular interest in that it is currently the FDA approved drug for use as a therapeutic agent in the treatment of transfusion induced iron overload associated with β thalassemia (Cooley's Anemia) [4, 5].

Microcrystalline ferrioxamine B, $[Fe(HDFB)]ClO_4 \cdot 5H_2O$ was prepared from purified samples of deferriferrioxamine B and ferric ammonium sulfate using an isolation and purification scheme similar to that found in the literature [6]. *Anal.* Calcd.: %C, 37.35; %H, 7.02; %N, 10.45. Found [7]: %C 37.87; %H, 6.18, %N, 10.07; $\lambda_{(max)}(\epsilon)$ 425 nm (2464 (30) M^{-1} cm^{-1}). Kinetics experiments were carried out at 25 °C, I = 2.0 ($NaClO_4$), over the $[H^+]$ range from 10^{-2} to 1.0 *M* using techniques and equipment previously described [8].

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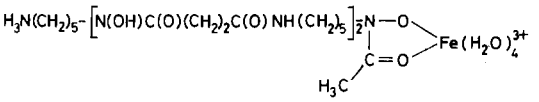
Four distinct reactions are observed for the aquation of $Fe(HDFB)^+$ over the $[H^+]$ range investigated. A detailed kinetic analysis [9] of the first three rapid reactions, along with a spectral characterization of the resulting intermediate product formed, allows us to conclude that the reactant for the final slow step of the aquation reaction is the bidentate bonded species shown in (II). This species dissociates slowly to yield the protonated form of



(II)

$Fe(H_3DFB)(H_2O)_4^{3+}$

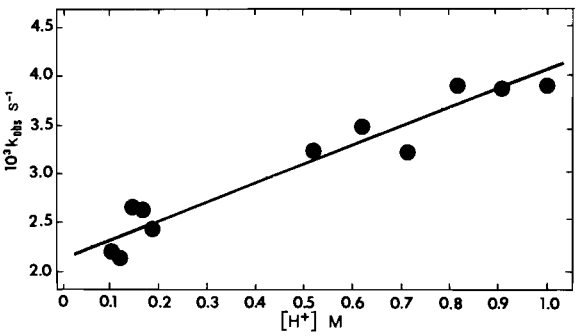


Fig. 1. Plot of pseudo first order aquation rate constant for $Fe(H_3DFB)(H_2O)_4^{3+}$ as a function of $[H^+]$ at 25 °C, I = 2.0 *M*. Each data point represents the average of 3 to 5 determinations.

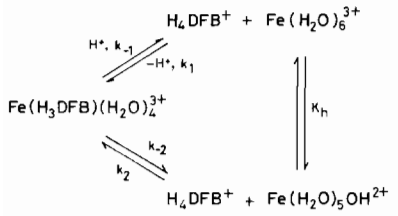
the free ligand, H_4DFB^+, and $Fe(H_2O)_6^{3+}$, as shown in eqn. (1). Figure 1 shows the influence of $[H^+]$ on the

$$Fe(H_3DFB)(H_2O)_4^{3+} + H_3O^+ \xrightarrow{H_2O} Fe(H_2O)_6^{3+} + H_4DFB^+ \quad (1)$$

experimentally observed pseudo first order aquation rate constant, k_{obs}. The rate law for the final stage of the aquation reaction is shown in eqn. (2), where k_{-1} = 1.93(0.20) × 10^{-3} M^{-1} s^{-1} and k_{-2} = 2.12(0.12) × 10^{-3} s^{-1}. The acid dependence shown in Fig. 1 is exactly analogous to that observed for the

$$\frac{-d[Fe(H_3DFB)(H_2O)_4^{3+}]}{dt} = (k_{-2} + k_{-1}[H^+])[Fe(H_3DFB)(H_2O)_4^{3+}] \quad (2)$$

aquation of a series of monohydroxamatoiron(III) complexes [8] $(Fe(R_1C(O)N(O)R_2)(H_2O)_4^{2+}$ and suggests the parallel path mechanism to products shown in Scheme A.



SCHEME A

The similarity between bidentate linked ferrioxamine B and the monohydroxamatoiron(III) complexes is most strongly emphasized when one considers the linear relationship between the variation in acid dependent and acid independent aquation rate constants with changes in the substituents R_1 and R_2 in $Fe(R_1C(O)N(O)R_2)(H_2O)_4^{2+}$. The corresponding rate constants for $Fe(H_3DFB)(H_2O)_4^{3+}$ aquation follow the linear relationship defined by the monohydroxamatoiron(III) complexes, as is shown in Fig. 2. This confirms our statement that the final species leading to $Fe(HDFB)^+$ aquation contains only one hydroxamate group bonded to iron(III), and furthermore that *this species behaves kinetically like the synthetic monohydroxamatoiron(III) complexes with respect to aquation.*

It is significant to note in Fig. 2 that $Fe(H_3DFB)(H_2O)_4^{3+}$ has lower acid dependent and independent aquation rate constants than any of the synthetic monohydroxamatoiron(III) complexes. The variations in acid dependent and independent aquation rate constant with changes in the R_1 and R_2 groups in $Fe(R_2C(O)N(O)R_2)(H_2O)_4^{2+}$ indicate that the R_2 substituent has a dominant influence (relative to R_1) in determining these trends. Furthermore, the origin of this substituent effect appears to be electronic with the more electron donating methyl group providing the lowest aquation rate constant [8]. The substituent corresponding to R_2 in the siderophore system is an alkyl group. Apparently the evolutionary process has developed a siderophore structure that minimizes the aquation rate by either path in Scheme A to enhance both the thermodynamic *and* kinetic stability of the iron complex.

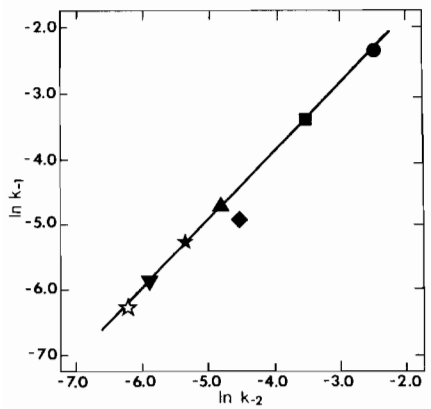


Fig. 2. Log-log plot for monohydroxamatoiron(III) (from Ref. 8) and ferrioxamine B acid dependent (k_{-1}) and acid independent (k_{-2}) aquation rate constants. ● $Fe(CH_3C(O)N(O)H)(H_2O)_4^{2+}$; ■ $Fe(C_6H_5C(O)N(O)H(H_2O)_4^{2+}$; ▲ $Fe(CH_3C(O)N(O)C_6H_5)(H_2O)_4^{2+}$; ◆ $Fe(C_6H_5C(O)N(O)C_6H_5)(H_2O)_4^{2+}$; ★ $Fe(CH_3C(O)N(O)CH_3)(H_2O)_4^{2+}$; ▼ $Fe(C_6H_5C(O)N(O)CH_3)(H_2O)_4^{2+}$; ☆ $Fe(H_3DFB)(H_2O)_4^{3+}$. All data collected at 25 °C, I = 2.0 ($NaClO_4$).

A detailed spectral and kinetic analysis of the entire ferrioxamine B aquation reaction [9] allows us to compute a value of 8×10^{-3} for the equilibrium constant for reaction (1) at 25 °C, I = 2.0 ($NaClO_4$). The rate constants for the reaction of deferriferrioxamine B, H_4DFB^+, with $Fe(H_2O)_6^{3+}$ and $Fe(H_2O)_5OH^{2+}$ may then be computed for these same conditions and found to be 2×10^{-1} M^{-1} s^{-1} and 2×10^2 M^{-1} s^{-1} respectively [10]. These rate constants are a factor of 4–20 smaller than the corresponding rate constants for the synthetic monohydroxamic acids ($R_1C(O)N(OH)R_2$), but are consistent with the trends established by them. This suggests a variable degree of hydroxamic acid ligand (synthetic monohydroxamic acid or deferriferrioxamine B) participation in the transition state, which is consistent with the associative character previously proposed for the monohydroxamatoiron(III) formation reactions [8]. Alternatively, charge effects and/or steric requirements associated with ferrioxamine B complex formation may cause some reduction in the formation rate constant.

These results serve to illustrate the validity of viewing the monohydroxamatoiron(III) complexes, $Fe(R_1C(O)N(O)R_2)(H_2O)_4^{2+}$, as models for the rate determining step of ferrioxamine B aquation and suggests similar comparisons with other hydroxamate siderophores.

Acknowledgements

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