Inorganica Chimica Acta, *138* (1987) 61–65

Kinetics and Mechanism of Aluminum(III)/Siderophore Ligand Exchange: Mono(deferriferrioxamine B)aluminum(III) Formation and Dissociation in Aqueous Acid Solution

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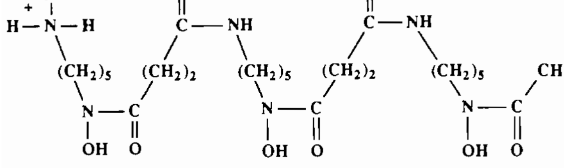
(Received February 17, 1987; revised March 25, 1987)

Abstract

The kinetics and mechanism of a linear trihydroxamic acid siderophore (deferriferrioxamine B, H_4DFB^+) ligand exchange with $Al(H_2O)_6{}^{3+}$ to form mono(deferriferrioxamine B)aluminum(III) ($Al(H_2O)_4H_3DFB)^{3+}$) have been investigated at 25 °C over the $[H^+]$ range 0.001–1.0 M and $I = 2.0$ M ($HClO_4/NaClO_4$) by ^{27}Al NMR. Kinetic results are consistent with $Al(H_2O)_4(H_3DFB)^{3+}$ formation and dissociation proceeding through a parallel path mechanistic scheme involving $Al(H_2O)_6{}^{3+}(k_1/k_{-1})$ and $Al(H_2O)_5(OH)^{2+}(k_2/k_{-2})$ where $k_1 = 0.13$ M^{-1} s^{-1}, $k_{-1} = 8.7 \times 10^{-3}$ M^{-1} s^{-1}, $k_2 = 2.7 \times 10^3$ M^{-1} s^{-1}, and $k_{-2} = 9.6 \times 10^{-4}$ s^{-1}. Relative complex formation rates at $Al(H_2O)_6{}^{3+}$ and $Al(H_2O)_5OH^{2+}$, and comparison with kinetic data for a series of synthetic hydroxamic acids, suggest that an interchange mechanism is operative. These results are also discussed in relation to kinetic data for the corresponding iron(III)–deferriferrioxamine B system.

Introduction

Isomorphous metal replacement of paramagnetic iron(III) by diamagnetic aluminum(III) in ferrichrome [1] and ferrioxamine B [2] has proven to be a successful technique by which to study the solution chemistry of these biologically important metal chelate complexes by NMR spectroscopy. Deferriferrioxamine B (**I**) is a microbially generated trihydroxamic acid siderophore whose biological function is to solubilize iron(III) from the environment and transport it to the cell, where it is then utilized for various processes essential to life [3–5].



I

Deferriferrioxamine B

Aluminum is the most abundant metal in the earth's crust, existing predominately as aluminates and aluminosilicates [6]. Due to the widespread occurrence of acid rain pollution, aluminum ores may be dissipated into the environment in increasing amounts as soluble cationic species capable of exhibiting toxic effects on aquatic wildlife [7–9]. Consequently, the availability of toxic aluminum to living organisms is ever increasing, and could disrupt life-essential processes at the cellular level via siderophore transport. The kinetics and mechanism of aluminum uptake and release by a naturally occurring siderophore is then of some significance. Deferriferrioxamine B is currently the US-FDA approved drug (Desferal®, Ciba-Geigy) for use as a therapeutic agent in the treatment of transfusion induced iron overload associated with β thalassemia (Cooley's Anemia) [10]. The deferriferrioxamine B complex with aluminum(III) is of particular interest in that DFB has been employed in chelation therapy to remove aluminum from patients suffering from dialysis encephalopathy, Alzheimer's disease, and other ailments associated with elevated levels of aluminum in the body [11, 12]. Herein we wish to report the kinetics and mechanism of mono(deferriferrioxamine B)aluminum(III), $Al(H_2O)_4(H_3DFB)^{3+}$, formation and dissociation in aqueous acid solution.

Experimental

Materials

Aqueous solutions were prepared using doubly distilled water. Aluminum nitrate (Fisher Scientific) was once recrystallized by preparing a concentrated solution of $Al(NO_3)_3 \cdot 9H_2O$ in a 50/50 mixture (*v*/*v*) of acetone/water and adding acetone (10^3-fold excess) until cloudiness persisted. Aluminum nitrate precipitated as a white microcrystalline powder upon refrigeration. Perchloric acid (70% Mallinckrodt) was

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standardized by titrating with 1 N NaOH (Fisher). Stock sodium perchlorate was standardized by passing an aliquot through a Dowex 50W-X8 20–50 mesh cation exchange column and titrating the liberated H^+ to the phenolpthalein end point. The methyl sulfonate salt of deferriferrioxamine B (Desferal mesylate®) was obtained as a generous gift from Ciba-Geigy Corporation.

^{27}*Al NMR Spectra and Kinetics*

Ligand exchange kinetics were studied in aqueous acid solution under pseudo first-order conditions with respect to deferriferrioxamine B concentration over the $[H^+]$ range 1.0–0.001 M at 25 °C and 2.0 M ionic strength ($HClO_4/NaClO_4/CH_3SO_3^-$). All solutions were 0.01 M in $Al(NO_3)_3 \cdot 9H_2O$ with the deferriferrioxamine B concentration being held constant over the $[H^+]$ range investigated. Deferriferrioxamine B concentration ranged from 0.10 M to 0.50 M.

^{27}Al NMR spectra were obtained using a JEOL Model FX-90Q spectrometer equipped with an omnitunable probe and a JEOL Model NM-TVS temperature controller. An observation frequency of 23.34802 MHz and a pulse width of 29.0 μs (60° pulse) were employed at a spectral width of 54 Hz. The zero-filling method (SAMPO 256, Point 8192) significantly increased the signal-to-noise ratio of each spectrum and reduced data accumulation time. The use of double precision (16 K memory) prevented memory overload and subsequent loss of accumulated data. All spectra were recorded after 800 scans.

The acquisition and analysis of kinetic data involved measuring the width at one half height of the ^{27}Al NMR resonance of $Al(H_2O)_6^{3+}$ in the absence (h_0) and in the presence (h_{ex}) of deferriferrioxamine B exchange at a given $[H^+]$. An observed pseudo first-order rate constant was obtained at conditions of slow exchange [13] by use of the equation: $k_{obs}\,(s^{-1}) = \pi(h_{ex} - h_0)$.

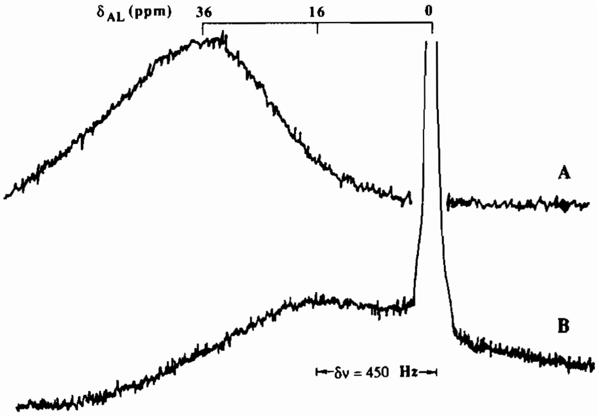
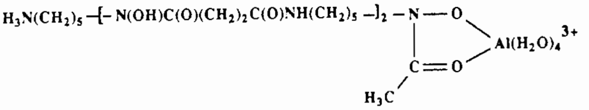


Fig. 1. ^{27}Al NMR spectra (A) $Al(HDFB)^+$ at 36 ppm; $[Al^{3+}]$ = 0.15 M, $[H_4DFB^+]$ = 0.15 M, $[H^+] = 10^{-8}$ M, 25 °C. (B) Coexistence of $Al(H_2O)_4(H_3DFB)^{3+}$ at 13 ppm and $Al(H_2O)_6^{3+}$ at 0 ppm, separation $\delta\nu$ = 450 Hz; $[Al^{3+}]$ = 0.15 M, $[H_4DFB^+]$ = 0.15 M, $[H^+] = 10^{-4}$ M, 25 °C.

Results

Figure 1(A) is an ^{27}Al NMR spectrum of an aqueous pH 8 solution containing $[Al^{3+}]$ = [deferriferrioxamine B] = 0.15 M. A single resonance ($\Delta\nu_{1/2}$ = 1140 Hz) is observed at 36 ppm downfield from an external $Al(H_2O)_6^{3+}$ reference standard. Previous work on the ^{27}Al NMR of aluminum(III) analogues of siderophore compounds [1(b), 14] supports the interpretation of this resonance being attributed to the formation of the hexadentate species, $Al(HDFB)^+$, in solution. The ^{27}Al NMR spectrum of the same solution at pH 4, exhibits two peaks (Fig. 1(B)), with one being a broad, low intensity resonance ($\Delta\nu_{1/2}$ = 1800 Hz) 16 ppm downfield from a much sharper, higher intensity hexaaquoaluminum resonance. Similar spectral changes have been observed for tris-acetohydroxamato)aluminum(III) in solution [1(b), 14], which support the interpretation of the 16 ppm resonance downfield from $Al(H_2O)_6^{3+}$ as being due to the formation of mono(deferriferrioxamine B) aluminum(III), $Al(H_2O)_4(H_3DFB)^{3+}$. Increasing the acidity of a deferriferrioxamine B aluminum(III) solution from pH 8 to pH 4 results in the protonation and dissociation of the hydroxamate groups from the Al^{3+} coordination sphere and replacement by H_2O ligands. Consequently, at pH 4 the predominant equilibrium in solution is the formation and dissociation of the bidentate species shown in **II**. Evidence for the structure of $Al(H_2O)_4(H_3DFB)^{3+}$ shown in **II** is based on a detailed kinetic analysis [15, 16] of the analogous iron(III) system. The fact that two separate resonances for $Al(H_2O)_4(H_3DFB)^{3+}$ and $Al(H_2O)_6^{3+}$ are observed ($\delta\nu$ = 450 Hz) indicates that the formation and dissociation of $Al(H_2O)_4(H_3DFB)^{3+}$ shown in eqn. (1) is slow relative to the ^{27}Al NMR time scale [13].



II

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

$$Al(H_2O)_6^{3+} + H_4DFB^+ \rightleftharpoons Al(H_2O)_4(H_3DFB)^{3+} + H_3O^+ + H_2O \qquad (1)$$

Values of $k_{obs}\,(s^{-1})$ for reaction (1) as a function of $[H^+]$ at various deferriferrioxamine B concentrations at 25 °C and I = 2.0 M are compiled in Table I and available as Supplementary Material. Figure 2 is

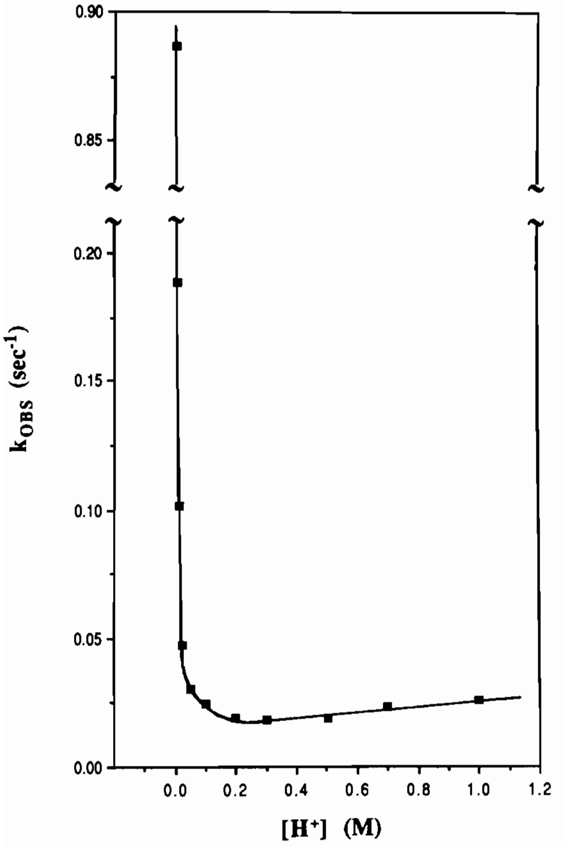


Fig. 2. Plot of experimentally observed rate constant, k_{obs} (s^{-1}), as a function of [H$^+$] for the $Al(H_2O)_4(H_3DFB)^{3+}$ system where $[H_4DFB^+] = 0.5$ M and $[Al^{3+}] = 0.01$ M; $T = 25$ °C; $I = 2.0$ M. Solid line represents a least squares fit of eqn. (2) to the experimental data.

a representative plot of some of these data. The solid line in Fig. 2 represents a least squares fit of the three-parameter eqn. (2) to the experimental data.

$$k_{obs} = a + \frac{b}{[H^+]} + c[H^+] \qquad (2)$$

The kinetic results are consistent with the following scheme.

$$Al(H_2O)_6^{3+} + H_4DFB^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Al(H_2O)_4(H_3DFB)^{3+} + H_2O + H_3O^+ \qquad (3)$$

$$\Big\Updownarrow Q_H$$

$$Al(H_2O)_5(OH)^{2+} + H_4DFB^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Al(H_2O)_4(H_3DFB)^{3+} + 2H_2O \qquad (4)$$

Scheme 1.

Due to the high pK_a for deferriferrioxamine B [17], reaction paths involving the free hydroxamic acid anion may be ignored over the [H$^+$] range investigated and therefore no proton ambiguity exists for this system.

When the mechanism shown in Scheme 1 is treated as a reversible process at the conditions $10[Al^{3+}]_{tot} \leqslant [H_4DFB^+]_{tot} = [H_4DFB^+]_{eq}$, then eqn. (5)

$$k_{obs} = k_1[H_4DFB^+]_{tot} + k_{-2} + k_2Q_H[H_4DFB^+]_{tot}/[H^+] + k_{-1}[H^+] \qquad (5)$$

for the experimentally observed rate constant, k_{obs}, may be derived. Equation (5) is of the same analytical form as eqn. (2) where $a = (k_1[H_4DFB^+]_{tot} + k_{-2})$, $b = k_2Q_H[H_4DFB^+]_{tot}$, and $c = k_{-1}$. Values for the coefficients a, b and c were obtained from a nonlinear squares fit of eqn. (6), a rearranged form of eqn. (2), to the data at different fixed [H$_4$DFB$^+$].

$$[H^+]k_{obs} = a[H^+] + b + c[H^+]^2 \qquad (6)$$

Values of k_1 and k_{-2} were obtained from the slope and intercept, respectively, of a plot of a *versus* [H$_4$DFB$^+$], k_2 from b ($= k_2Q_H[H_4DFB^+]$ where $Q_H = 3.3 \times 10^{-6}$ M [6]), and k_{-1} directly from c. The calculated microscopic rate constants corresponding to Scheme 1 are as follows: $k_1 = 1.3 \times 10^{-1}$ M^{-1} s^{-1}; $k_{-1} = 8.7 \times 10^{-3}$ M^{-1} s^{-1}; $k_2 = 2.7 \times 10^{3}$ M^{-1} s^{-1}; and $k_{-2} = 9.6 \times 10^{-4}$ s^{-1}.*

Discussion

The complex formation rate constants k_1 and k_2 are of similar magnitude to literature values for Al_{aq}^{3+} substitution reactions with monodentate ligands [18–22]. This suggests that hydroxamate ring closure is rapid in the formation of the structure **II** via reaction (1). Complex formation rates reported here are equivalent to those reported for a series of synthetic hydroxamic acids, $R_1C(O)N(OH)R_2$ [14]. This fact, along with the fact that the ratio $k_2/k_1 \sim 10^4$ is in rough agreement with the expected relative rates of H$_2$O exchange at $Al(H_2O)_6^{3+}$ [23] and $Al(H_2O)_5OH^{2+}$, suggests that complex formation is dominated by water exchange energetics and that the mechanism for ligand substitution is an interchange I process [14]. There is indirect evidence of the possibility of some associative character to reaction (1) with synthetic hydroxamic acids [14], which would also apply to the deferriferrioxamine B system reported here.

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*Standard deviations for k_1, k_{-1}, k_2 and k_{-2} are 1.5×10^{-3}, 1.0×10^{-4}, 5.3×10^{1} and 4.6×10^{-4} respectively. k_{-2} was obtained from the intercept of a plot of coefficient a from eqn. (2) *versus* [H$_4$DFB$^+$] where the correlation coefficient = 0.999.

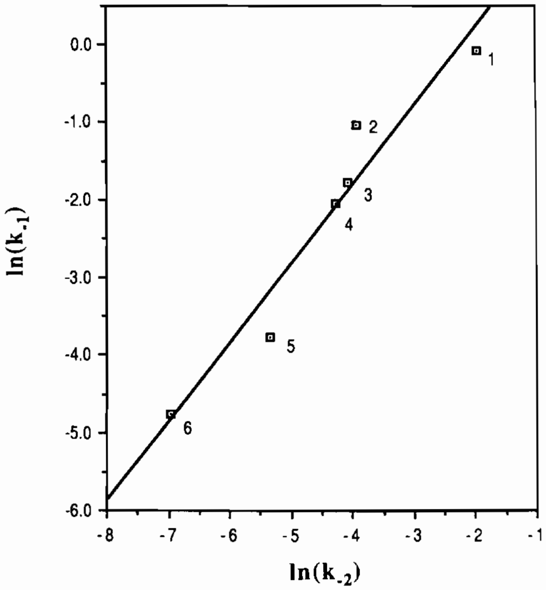

Fig. 3. Plot of the natural logarithm of the acid dependent aquation rate constant, ln(k_{-1}), as a function of the acid independent rate constant, ln(k_{-2}), for $Al(H_2O)_4(H_3DFB)^{3+}$ and a series of five complexes with synthetic hydroxamic acid ligands, $Al(H_2O)_4(R_1C(O)N(O)R_2)^{2+}$, from ref. 14. Numbered data points correspond to synthetic hydroxamic acids ($R_1C(O)N(OH)R_2$) as follows: 1. $R_1 = C_6H_5$, $R_2 = H$; 2. $R_1 = CH_3$, $R_2 = H$; 3. $R_1 = CH_3$, $R_2 = 4\text{-}CH_3C(O)C_6H_4$; 4. $R_1 = CH_3$, $R_2 = C_6H_5$; 5. $R_1 = CH_3$, $R_2 = 4\text{-}CH_3C_6H_4$; 6. deferriferrioxamine B (H_3DFB). Slope = 1.01 (0.13), correlation coefficient = 0.94.

Dissociation rate constants, k_{-1} and k_{-2}, for Al(III)–deferriferrioxamine B $Al(H_2O)_4(H_3DFB)^{3+}$, however, are significantly smaller than observed for a series of synthetic hydroxamic acids [14]. This is shown graphically in Fig. 3 where ln k_{-1} is plotted against ln k_{-2} for a series of synthetic hydroxamic acids and deferriferrioxamine B. This demonstrates that all of these reactions proceed by the same reaction scheme [24] and that the siderophore system forms the kinetically most stable complex. A similar linear correlation between ln k_{-1} and ln k_{-2} was observed for the corresponding iron(III) complexes, with ferrioxamine B representing the kinetically most stable complex [16]. Arguments have been presented previously which point out that changing the hydroxamate N-substituent to an alkyl group minimizes the iron(III) complex dissociation rate constants [25, 26]. We would expect the same to be true for aluminum(III) complexes and structure **II** illustrates the fact that in $Al(H_2O)_4(H_3DFB)^{3+}$ the N-substituent is a substituted alkyl group.

A direct comparison between the iron(III) and aluminum(III) deferriferrioxamine B systems is of some environmental relevance. An experimental stability constant for hexadentate complex formation is available for ferrioxamine B [17, 27], but not for the aluminum(III) analogue. Schwarzenbach, *et al.* [28]*, however, have estimated that the hexadentate aluminum(III)–deferriferrioxamine B complex would be seven orders of magnitude less stable than ferrioxamine B. Based on results reported here and elsewhere [15, 16] this is a manifestation of both the fact that dissociation rate constants, (k_{-1}, k_{-2}) are larger for Al(III) and complexation rate constants (k_1, k_2) are larger for Fe(III). Complexation rates appear to be dominated by water exchange energetics for both metal ions. Dissociation rates may reflect the higher electronegativity of Fe(III) which results in a greater degree of covalent character for the dissociating Fe–O=C< bond. Based on the kinetic data reported here we can calculate a stability constant for $Al(H_2O)_4(H_3DFB)^{3+}$ formation (reaction (1)) which is only 10^1 times less than the corresponding value for $Fe(H_2O)_4(H_3DFB)^{3+}$ determined from spectrophotometric measurements [15]. Since we would not expect this difference in stability constants to become greater in subsequent chelation steps to form $M(H_2O)_2(H_2DFB)^{2+}$ and $M(HDFB)^+$ (M = Al(III), Fe(III)), we predict that the difference in overall stability constants between the fully chelated hexadentate complexes $Fe(HDFB)^+$ and $Al(HDFB)^+$ to be significantly less than the seven orders of magnitude previously predicted. This higher than expected [28, 29] stability for the Al(III)–deferriferrioxamine B system suggests that high environmental Al(III) concentrations may influence siderophore mediated iron transport through competition.

Supplementary Material

A list of observed first order rate constants at various $[H^+]$ and $[H_4DFB^+]$ is available from the authors on request.

Acknowledgements

Acknowledgement is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. We also thank the Ciba-Geigy Corporation for their generous gift of the methanesulfonate salt of deferriferrioxamine B (DESFERAL®).

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*Estimate based on comparison between the tris complex stability constants of iron(III) and aluminum(III) with acetohydroxamic acid reported by same authors in ref. 27.

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