Crystal Structure and Redox Behavior of a Novel Siderophore Model System: A Trihydroxamato−Iron(III) Complex with Intra- and Interstrand Hydrogen Bonding Networks

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Microorganisms produce and release chelating compounds known as siderophores for cellular sequestration of iron.[1] Crystal structures of the iron(III) complexes with natural siderophores containing three hydroxamate groups have been presented.[2,3] In the crystal structures of the ferrichrome-type siderophore, the existence of intramolecular hydrogen bonds was revealed.[2,3] It has been deduced that such intramolecular hydrogen bonds may contribute to the stabilization[4−6] and chiral preference of the complex.[4,5] The redox behavior of iron siderophore complexes is another important aspect of siderophore function because the release of iron ion in the cells proceeds through reduction of the iron(III) ion.[6a] Under physiological conditions, however, it is difficult to reduce the complex using typical biological reducing agents such as NAD(P)H, because of their larger negative redox potentials.[6b,7] The redox potential of iron siderophore complexes increases at acidic pH,[7] and the introduction of multiple hydrogen bonds tends to lower the pH locally in the vicinity of the coordinating atoms.

We have synthesized a novel tripodal hydroxamate ligand, tris-[2-{(*N*-acetyl-*N*-hydroxy)glycylamino}ethyl]amine (TAGE), which was designed so that intra- and interstrand hydrogen bonding networks are formed in the complex. Here, we describe the crystal structure, stability, and redox behavior of the iron(III) complex with TAGE.

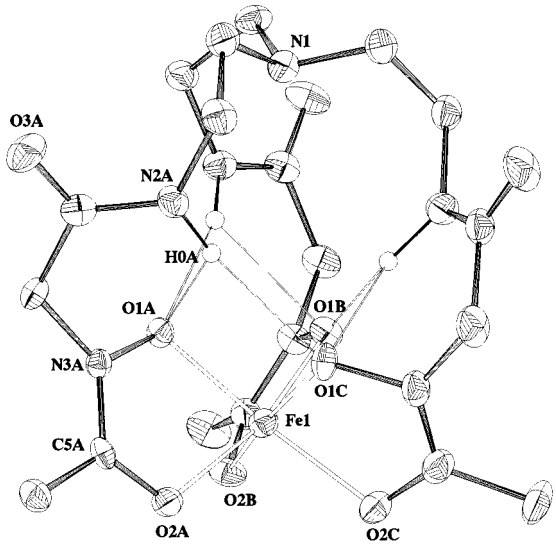

Figure 1. Crystal structure of **1** showing the atom-numbering scheme. Selected bond lengths (Å) and angles (deg): Fe−O(1A) = 1.973(4), Fe−O(1B) = 1.961(4), Fe−O(1C) = 1.976(4), Fe−O(2A) = 2.010(4), Fe−O(2B) = 2.021(4), Fe−O(2C) = 2.036(4), O(1A)−Fe−O(2A) = 79.3(2), O(1B)−Fe−O(2B) = 79.0(2), O(1C)−Fe−O(2C) = 78.4(2). Intra- and interstrand hydrogen bond distances (Å): N(2A)···O(1A) = 2.83, N(2B)···O(1B) = 2.92, N(2C)···O(1C) = 2.89, N(2A)···O(1B) = 3.25, N(2B)···O(1C) = 3.29, N(2C)···O(1A) = 3.06.

The ligand TAGE was prepared according to a modified literature method,[8] and the iron(III) complex of TAGE (**1**) was obtained by reaction of Fe(acetylacetonato)$_3$ with TAGE in a methanol solution.[9] A deep-red colored crystal of **1** was employed for a single-crystal X-ray diffraction analysis.[10] As shown in Figure 1, the iron atom has a distorted octahedral geometry with three hydroxamate groups of TAGE and the overall structure is twisted around a pseudo-3-fold axis.

Average bond lengths and angles between the iron(III) atom and the coordinating aminohydroxyl O(N) and carbonyl O(C) atoms, Fe−O(N) = 1.970(4) Å, Fe−O(C) = 2.022(4) Å, and O(N)−Fe−O(C) = 78.9°, respectively, are quite comparable to those for natural trihydroxamate siderophores.[2,3] The significantly shorter Fe−O(N) bond, compared to the Fe−O(C) bond, may be explained to be due to the negative charge localized on the deprotonated aminohydroxyl oxygen. These findings clearly imply that the iron(III) ion for **1** is located at an optimum position similar to the positioning of iron ions in natural iron siderophores.[2,3]

As was expected from the design concept of the ligand TAGE, the intra- and interstrand hydrogen bonding networks were formed in the crystal structure of **1**. All the amide N−H bond vectors are directed toward the coordinating aminohydroxyl oxygens with average N···O (H···O) distances of 2.88 Å (2.14 Å) within the same strand. Such intrastrand hydrogen bonding networks were

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(9) Elementaly analysis of **1**. Found: C, 37.60; H, 5.60; N, 16.99. Calcd for $C_{18}H_{30}N_7O_9Fe\cdot 2H_2O$: C, 37.25; H, 5.91; N, 16.89.
(10) Crystal data for **1**: $FeC_{18}H_{30}N_7O_9\cdot H_2O$, M = 562.34, monoclinic, $P2_1/n$, a = 10.5197(7) Å, b = 17.679(1) Å, c = 13.7957(9) Å, β = 104.538(6)°, Z = 4, V = 2483.6(3) Å^3, D_c = 1.504 g cm^{-3}, μ(Mo Kα) = 6.72 cm^{-1}, F(000) = 1180.0, R = 0.051, R_w = 0.042. A total of 5208 unique reflections were collected on an Enraf Nonius CAD4-EXPRESS four-circle diffractometer, of which 2353 reflections with $I > 3\sigma(I_o)$ were used in the structure analysis and refinement using the teXsan program system. Absorption correction was applied.
(11) The cyclic voltammogram of **1** was measured under the following condition: [**1**] = 1 mM, 50 mM Tris-HClO$_4$ buffer at pH 7.3, 1 M NaClO$_4$ or 0.1 M tetra-*n*-buthyllammonium tetrafluoroborate as supporting electrolyte, glassy carbon working electrode, saturated calomel reference electrode (SCE), Pt wire supporting electrode, scan rate 10 or 100 mV/s. The potential values, which were measured as the SCE scale, were converted to the NHE scale by addition of +242 mV.
(12) The pM value is defined as −log [Fe(H$_2$O)$_6$], which is calculated by using the stability constants of the complex (log β) and the protonation constants of the ligand (pK_n) under given conditions (at pH 7.4, [Fe(III)]$_{tot}$ = 1 μM, and [TAGE]$_{tot}$ = 10 μM). See also ref 6c.

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also detected in the crystal structures of the natural siderophores (ferrichrome-type complexes (2.73−2.81 Å for N···O).[2,3] Furthermore, the amide hydrogens of **1** also are intramolecularly bound to the coordinating aminohydroxyl oxygens between the interstrands with average N···O (H···O) distances of 3.20 Å (2.13 Å). The hydrogen bonding networks are likely to play an important role in shielding the central metal atom from the outer sphere and in stabilizing the ligand. This indicates that TAGE protects the metal ion from hydrolysis.

The cyclic voltammogram of **1**[11] in an aqueous solution showed a quasi-reversible redox wave corresponding to an Fe^{III}/Fe^{II} couple, the half-wave potential ($E_{1/2}$) of which was observed at −230 mV vs NHE with a peak separation of 74 mV. Recently it has been reported that the redox potentials corresponding to Fe^{III}/Fe^{II} for tris(hydroxamato)iron(III) complexes in aqueous solution exhibit a linear correlation with their pM values.[6c,7,12] The redox potential of the natural siderophore ferrichrocin has a pM value of 26.5 and a redox value of −412 mV vs NHE[6b,13] and thus appears to follow the correlation. The potential determined for **1** was −450 mV, significantly more positive than the value predicted from its pM value of 25.0,[14] even though the metal ion coordination structure and the pM value of **1** are almost the same as those for natural siderophores[2,3] except for the existence of hydrogen bonding networks in **1**. Taking into account the $E°$ values for the redox couples $Fe^{III}L/Fe^{II}L$ ($E°_{complex} = -230$ mV for L = TAGE) and Fe^{III}/Fe^{II} ($E°_{aq} = 732$ mV)[7] and the stability constant of **1** ($\log \beta^{III} = 28.7$),[14] the stability constant of iron(II)−TAGE (β^{II}) can be calculated from eq 1 and gives $\log \beta^{II} =$ 12.4. This result suggests that the iron(II) complex of TAGE is more stable than those of other trihydroxamate complexes (Table 1).

$$E^0_{complex} = E^0_{aq} - 59.15 \log(\beta^{III}/\beta^{II}) \quad (1)$$

To examine the influence of hydrogen bonding networks on redox potential, the iron(III) complex of tris[2-{3-(*N*-acetyl-*N*-hydroxyamino)propylamido}ethyl]amine (**1′**) was prepared as an analogue of **1** lacking the hydrogen bonding networks. Redox potentials for **1′** were measured in methanol solution because of poor stability in aqueous solution. The peak potentials of **1′** exhibited a lower value of −540 mV vs NHE[11] (although only a cathodic wave was given), which is lower also in comparison with that of **1** ($E_{1/2} = -360$ mV vs NHE, $\Delta E = 110$ mV)[11] in methanol solution. Consequently, a larger positive shift of the potential for **1** suggests that *the intra- and interstrand hydrogen bonding networks* stabilize both the iron(II)− and iron(III)−TAGE complexes by tight fixation of the metal ion with TAGE ligand. It is also interesting that complex **1** may be easily reduced by biological reducing agents such as NAD(P)H under physiological conditions without any hydrolytic decomposition of the ligand framework.

Table 1. Redox Potentials, pM Values, and Stability Constants

iron complex	$E_{1/2}$[a]	pM	$\log \beta^{III}$	$\log \beta^{II}$
FeTAGE (**1**)[b]	−230	25.0	28.7	12.4[c]
ferricrocin[d]	−412	26.5	30.4	11.0[c]
$Fe(NMeAHA)_3$[e]	−348	16.2	29.4	11.1[c]

[a] In mV vs NHE. [b] This work. [c] Overall stability constant for the Fe(II)−hydroxamate complex were calculated from eq 1. [d] Reference 6b. [e] NMeAHA; *N*-methyl-acetohydroxamic acid (ref 7).

In conclusion, we have synthesized the first iron(III) complex of a novel artificial tripodal trihydroxamate with multi-intramolecular hydrogen bonds and shown that intramolecular hydrogen bonding networks generated in the tris(hydroxamato)-type complex not only contribute to tight binding and shielding of the iron(III) atom from the outer sphere but also provide control of the redox potential of the central metal ion. Detailed investigations of the influence of hydrogen bonding networks on transportation of the iron(III)−TAGE complex into bacterial cells are now under way.

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Supporting Information Available: Tables listing positional parameters and *B*(eq), anisotropic displacement parameters, and intramolecular distances and bond angles involving the non-hydrogen atoms and a figure depicting the cyclic voltammogram of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) The pM value of the iron(III)−TAGE system was calculated by using the stability constant of **1** ($\log \beta = 28.7$) and the protonation constants (pK_a) of TAGE ($pK_1 = 9.79$, $pK_2 = 8.95$, $pK_3 = 8.06$) under the same conditions as in ref 12. Using ref 6c, the pK_as of TAGE and the formation constant of **1** were determined by the potentiometric titration and the spectrophotometric measurement of the competition reaction with EDTA, respectively.