Preorganization of Ferric Alcaligin, Fe_2L_3. The First Structure of a Ferric Dihydroxamate Siderophore

Zhiguo Hou, Christopher J. Sunderland, Takayuki Nishio, and Kenneth N. Raymond*

Department of Chemistry
University of California, Berkeley
Berkeley, California 94720

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Siderophores are microbial iron transport agents that are produced when the organism is iron deficient. Their medical significance is due to iron being a limiting nutrient to bacterial growth, hence siderophores often are determinants of bacterial pathogenicity.[1−4] Siderophores form strong and selective complexes of Fe(III) which are usually taken up via membrane receptors. The recognition and transport of these complexes are generally stereoselective, primarily recognizing the metal ion coordination geometry.[3,5] Most siderophores are hexadentate, completely encapsulating octahedral Fe(III). In order to encapsulate completely the metal ion, a dihydroxamate siderophore (represented by L) must form minimally a binuclear metal complex with a stoichiometry of Fe_2L_3. An early example is provided by rhodotorulic acid (**RA**, Figure 1), the linear dihydroxamate siderophore of *Rhodotorula pilimanae*, which forms a complex of stoichiometry Fe_2L_3.[6,7] What is the structure of such a complex? As shown in Figure 1 there are two topologies which can equally conform to the Fe_2L_3 stoichiometry; a related system, dimerum acid, was proposed to form a monobridged dimer,[8] however the **RA** complexes were assigned tri-bridged helical structures as found in a synthetic hydroxypyridonate analog that was fully structurally characterized.[9] These can be regarded as the first helicates, a class of supramolecular structures that have been recent synthetic goals.[10,11] We report here the first example of the alternative mono-bridged structure (Figure 1) as found in the Fe_2L_3 complex of alcaligin.

Alcaligin (**AG**) is a 20-membered C_2 symmetrical macrocyclic dihydroxamate (Figure 1) that was first isolated from a marine algae, *Alcaligenes denitrificans* subsp. *xylosoxydans.*[12] It very recently was found to be the siderophore for two mammalian pathogens, *Bordetella pertussis* (which causes whooping cough in humans) and *B. bronchiseptica* (which causes kennel cough in dogs and a similar disease in swine).[13]

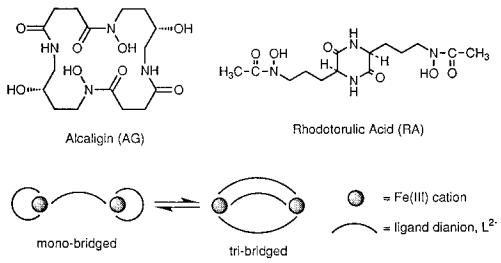

Figure 1. The bis-hydroxamate siderophores alcaligin and rhodotorulic acid and schematic diagrams of their possible dimeric six-coordinate iron complexes.

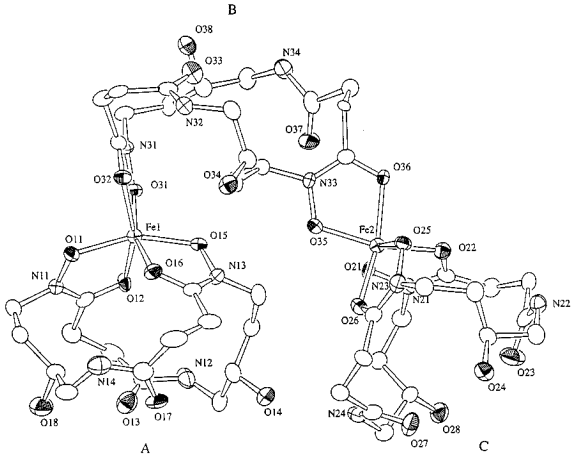

Figure 2. The structure of the Fe_2AG_3 complex showing the three different ligand fragments: terminal (**A** and **C**) and bridging (**B**). The iron atoms are labeled as Fe1 and Fe2.

With other recently characterized compounds[14,15] they represent a new class of macrocyclic dihydroxamate siderophores.

Thermodynamic studies of **RA**[7] and **AG**[16] (Table 1) show that both form Fe_2L_3 species at neutral pH while a tetradentate monomeric complex, FeL^+, predominates at pH 2. For the reactions $Fe^{3+} + L^{2-} = FeL^+$ (K_1) and $2FeL^+ + L^{2-} = Fe_2L_3$ (K_2), the value of the stability constant K_1 ($10^{23.5(2)}$) for **AG** is about 100 times that for **RA** ($10^{21.6}$), while K_2 is correspondingly about 100 times weaker ($10^{17.7(2)}$ vs $10^{19.8}$). It will be seen that these values devolve directly from the stereochemistry; alcaligin is the first preorganized siderophore.

An X-ray quality crystal of the 2:3 ferric complex of **AG** (Fe_2AG_3) was obtained from a solution of H_2O/CH_3CN. As shown in Figure 2, the structure of Fe_2AG_3 adopts a monobridged binding mode, with a U-shaped molecular conformation.[17] The bridging ligand twists significantly from C_2 molecular symmetry. Each Fe^{3+} is coordinated in a pseudooctahedral environment provided by three hydroxamates. The absolute configuration is confirmed by the structure analysis[17] and with the determination of structure by synthesis.[18] The

* Author to whom correspondence should be submitted.

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(16) The protonation and stability constants of alcaligin were determined as described in the following: Hou, Z.; Whisenhunt, D. W. J.; Xu, J.; Raymond, K. N. *J. Am. Chem. Soc.* **1994**, *116*, 840−846. Hou, Z. Ph.D. Dissertation, University of California, Berkeley, 1995.
(17) The synthesis and structural solution of the complex $Fe_2(AG)_3$ are given in the supporting information. X-ray diffraction data are as follows: $Fe_2(AG)_3 \cdot 25H_2O$: Mo Kα radiation; $T = 125$ K; space group $P2_12_12_1$; $a = 13.3374(4)$ Å; $b = 16.1879(5)$ Å; $c = 37.886(1)$ Å; $Z = 4$; unique data ($I > 3.0(I)$, 5512, $R(Rw) = 0.053$ (0.068)). The absolute configuration was determined by the Bijvoet technique (supporting information).
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Table 1. Thermodynamic Comparison of Alcaligin with Rhodotorulic Acid

	protonation constants		formation constants with Fe^{3+}			
ligand	log K_{a1}	log K_{a2}	log K_1	log K_2	log β_{230}	pM[a]
alcaligin	9.42(5)	8.61(1)	23.5(2)	17.74(22)	64.66(4)	23.0
rhodotorulic acid[b]	9.44(3)	8.49(3)	21.6	19.8	62.3	21.9

[a] pM = $-\log[Fe^{3+}]$ at pH 7.4 with total ligand concentration = 10^{-5} M and total iron concentration = 10^{-6} M. [b] From ref 7.

Table 2. Metrical Parameters of the Ferric Alcaligin Complex and Other Ferric Hydroxamate Complexes (from Ref 5)

	ferric alcaligin				
parameter	Fe_1	Fe_2	ferrichrome	ferrichrome A	ferrioxamine E
Fe−O(C) (Å)	2.045	2.046	2.034	2.033	2.055
Fe−O(N) (Å)	1.981	1.974	1.983	1.980	1.953
O···O (Å)	2.526	2.532	2.534	2.527	2.549
normalized bite[a]	1.25	1.26	1.26	1.26	1.27
trigonal twist angle (deg)	33.0	33.1	42.9	41.4	45.1
O−Fe−O (deg)	77.7	78.0	78.2	78.0	78.9

[a] Normalized bite: 2 sin(θ/2), θ = O−Fe−O angle.

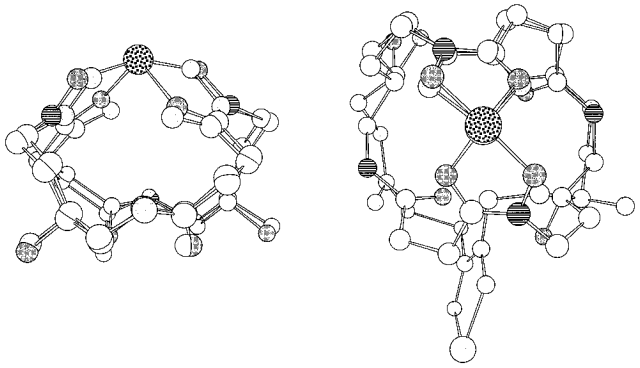

Figure 3. Comparisons of structure which show the preorganization of alcaligin for metal binding. (a, left): The structure of the free ligand (the lighter) is overlaid on one of the terminal ligands (**C**, the darker) of the iron complex. The rms deviation in atom positions is 0.227 Å. (b, right): The structure of the central (**B**, the lighter) and terminal (**A**, the darker) ligands in **$Fe_2(AG)_3$** are overlaid. The central ring must twist from its ground state structure, which is preorganized to bind a common metal center, in order to bridge two metal ions.

metal centers are each in a Λ absolute configuration, and are separated by 7.44 Å. In general, the two chelate centers are not only quite similar to each other, but also are consistent with other characterized ferric hydroxamate structures (Table 2).[19] However, the twist angles (33.1°, 33.0°) of the structure are about 10° smaller than that of the other ferric tris-hydroxamate siderophore complexes and the complexes are trans isomers, the second such example observed among 15 structures.[20] Both these unusual features are due to the small ligand ring size and resultant structural constraints of the ligand.

The conformational rigidity of **AG** makes the two terminal bis-bidentate fragments essentially identical. For the 20 atoms of the macrocyclic ring the rms deviation between these two fragments is 0.232 Å. Figure 3a shows the overlay of one terminal fragment (darker) of **Fe_2AG_3** with the free ligand structure (lighter);[12] the rms deviation from the free ligand (again of the 20 ring atoms) is 0.274 Å for the terminal fragment labeled **A** and 0.227 Å for that labeled **C**. Figure 3b shows the conformation of the bridging ligand (**B**). While half of the molecular fragment maintains the same conformation as that of the terminal (**A** and **C**) fragments, the other half deforms significantly in order to bridge two metal centers. The similarity of the free ligand conformation of iron chelating fragments of **Fe_2AG_3** is remarkable and can be viewed as resulting from the inherent structural features of **AG**. That is, **the free ligand is highly preorganized for binding Fe^{3+}.**

The **Fe_2AG_3** structure is unique in being the first Fe_2L_3 siderophore structure crystallographically characterized and in providing the first example of the mono-bridged structural alternative to the triple helicates. The latter topology has been assigned to the complexes of rhodotorulic acid and its synthetic analogs. Many recent studies have indicated that both stereochemical preference of metal ions and the ligand spacer separating two binding groups are essential for the formation of a triple-helix supramolecular structure.[21−23] The ground state conformation of the alcaligin macrocyclic ring is inconsistent with helix formation.

The crystal structure provides a ready explanation for the differences seen in the stability constants for alcaligin and rhodotorulic acid. The preorganization of the alcaligin ring puts both chelate rings in the correct position for the metal complex. This results in a 100-fold increase in the stability of the complex compared to the flexible rhodotorulic acid. However, this conformational stability is a liability in the trimer complexation reaction represented by K_2, with a resultant 100-fold decrease in relative stability. Overall the trimer formation involves K_1 twice and K_2 once, such that the alcaligin trimer is 100 times as stable as that of rhodotorulic acid. Alcaligin is a siderophore whose geometry is optimized for initial complexation of iron, a key to its role as a pathogen growth factor.

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Supporting Information Available: Experimental details of the synthesis of $Fe_2(AG)_3$, crystallographic structure determination, and tables of bond distances, angles, positional coordinates, and thermal parameters are available (14 pages). Ordering information is given on any current masthead page.

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