Spectral and Structural Characterization of Two Ferric Coordination Modes of a Simple Bis(catecholamide) Ligand: Metal-Assisted Self-Assembly in a Siderophore Analog

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Due to the insolubility of iron hydroxide, bacteria have developed sequestering agents, siderophores, to scavenge the iron required for life.[1] Given the diversity of environments in which microbes exist, a vast arsenal of siderophores has evolved, with catecholate and hydroxamate predominating as the bidentate ligating subunits.[2] A large number (12) of bis(catecholamide) siderophores have been identified, the majority in the past 2 years.[3−11] These siderophores are intriguing because they lack the six requisite atoms to fully ligate a single iron atom. Consequently, a discrete ferric complex ligated exclusively by these siderophores inherently must contain multiple ligands and metals. This complex would demonstrate a biologically important system of metal-assisted self-assembly,[12,13] as has been demonstrated with an analog of rhodotorulic acid.[14] The bis-(catecholamide) siderophores, while diverse in organic framework, may utilize similar energetic interactions to assemble a common structural form of metal complex. In order to potentially define the assembly process of such siderophores, a simple analog has been synthesized and the iron(III) complexes have been characterized.[15]

The bis(catecholamide) ligand (ethylcam), **1**, used in this study consists of two catecholamide units linked by an ethyl bridge, which is flexible and allows multiple configurations of the two catecholamide subunits but is short enough to prevent tetradentate ligation of a single metal. Tetradentate, square-planar ligation of Ni^{2+} by a bis(catecholamide) ligand with a pentane spacer has recently appeared.[16] Basic aqueous solutions of various Fe:**1** ratios have readily distinguishable deep red versus blue-red colors, suggesting the formation of at least two distinct types of metal complexes.

The reaction of 3 equiv of **1**-H_4 with 2 equiv of Fe^{3+} and 12 equiv of base (3:2:12) yields deep red aqueous solutions characteristic of ferric tris(catecholamides)[17] (λ_{max} = 488 nm, ϵ/Fe = 4700). This complex is structurally characterized[18] as a hexaanionic dinuclear triple helix, $[Fe_2(\mathbf{1})_3]^{6-}$ (Figure 1). The complex is effectively D_3-symmetric, but only a single 2-fold axis is crystallographically imposed. All three ethyl backbones have staggered conformations with the amide nitrogens *anti* to each other (N−C−C−N torsional angles: 174, 174, 178°). One purpose of this conformation is presumably to maximize the distance between the negatively charged coordination spheres. The two catecholamide planes within each ligand are oriented nearly mutually perpendicularly (95, 95, 97°). The structure of $[Fe_2(\mathbf{1})_3]^{6-}$ is similar to the gallium dinuclear triple helix $[Ga_2(\mathbf{2})_3]^{6-}$ (**2** = *N,N′*-bis(2,3-dihydroxybenzoyl)-(2*R*,4*R*)-diaminopentane), recently reported,[19] except that the catecholamide bridging unit in the iron structure is shorter and consequently the metal centers are positioned closer together (10.0 Å versus 10.8 Å). The twist angles[20] in $[Fe_2(\mathbf{1})_3]^{6-}$ are smaller than those in the gallium structure (Fe, 37, 37°; Ga, 44, 48°), consistent with the difference in bonding between iron and gallium,[21] but $[Fe_2(\mathbf{1})_3]^{6-}$ has a much larger helical twist[22] of approximately 80° versus 60° for $[Ga_2(\mathbf{2})_3]^{6-}$. The larger helical twist results from the shorter bridge between the catecholamides. This apparent paradox is resolved upon examining the geometric constraints of a metal−catecholamide subunit and the number of remaining rotational degrees of freedom in the ligand. Each individual catecholamide subunit, from the catecholate through the first carbon of the bridge, is planar due to strong hydrogen-bonding between the amide proton and the *ortho*-hydroxylate.[23,24] The imposed planarity leaves three rotational degrees of freedom in free, deprotonated **1**. The additional constraint that the ligand bind two metals as a triple helix severely reduces all of these degrees of freedom and precludes kinking or bending of the helical strands of $[Fe_2(\mathbf{1})_3]^{6-}$. Consequently, a helical pitch of 60° that would perfectly stagger the coordination spheres to minimize electrostatic repulsions[19] is unattainable. In contrast, the additional carbon atom in the bridge of **2** provides a further degree of freedom that allows the helical strands to

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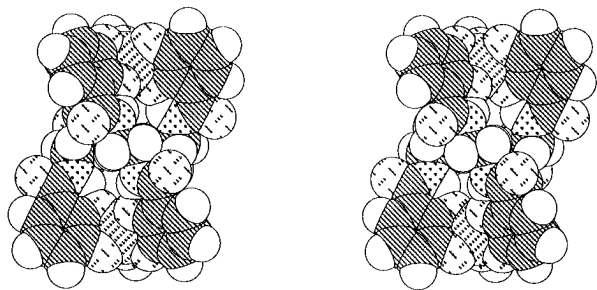
Figure 1. Space-filling stereoview of $[Fe_2(\mathbf{1})_3]^{6-}$ perpendicular to the pseudo-3-fold axis, down the crystallographic 2-fold axis.
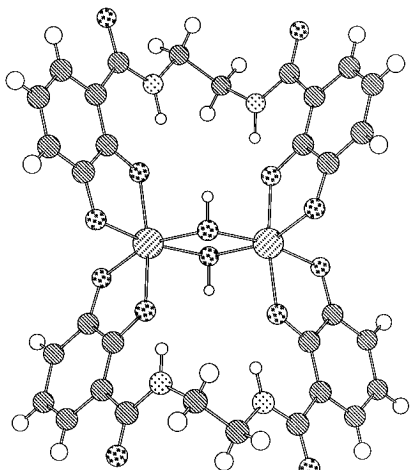
**Figure 2.** Ball and stick view of $[Fe_2(\mathbf{1})_2(OH)_2]^{4-}$ perpendicular to the pseudo-2-fold axis.

bend. Such a bend, readily obvious in the helical strands of $[Ga_2(\mathbf{2})_3]^{6-}$, permits the complex to adopt a 60° helical twist.

The reaction of $\mathbf{1}$-H_4 with Fe^{3+} and base in a 2:2:10 ratio yields blue-red aqueous solutions (λ_{max} = 502 nm, ϵ/Fe = 3400). This complex is now structurally characterized,[25] as a bis(μ-hydroxo) dimer, $[Fe_2(\mathbf{1})_2(OH)_2]^{4-}$ (Figure 2). In this novel tetraanionic dimer, each ligand is strapped between the two metals. Previous structurally-characterized bis(μ-hydroxo)iron-(III) dimers,[26–28] have all been neutral with each individual ligand bound to a single metal. The larger Fe–Fe distance of $[Fe_2(\mathbf{1})_2(OH)_2]^{4-}$ (3.196 Å vs average separation in the neutral dimers of 3.110 Å) reflects the charge difference of the coordination spheres. Surprisingly, solution molar susceptibility measurements at room temperature of $[Fe_2(\mathbf{1})_2(OH)_2]^{4-}$ in

acetonitrile and water do not demonstrate magnetic coupling.[29] This result is consistent with the single, strong g = 4.2 signal in the EPR spectra.[30] The two irons of $[Fe_2(\mathbf{1})_3]^{6-}$ are magnetically-uncoupled in solution, as anticipated from the large separation between the irons in the crystal structure (10.0 Å).

The $[Fe_2(\mathbf{1})_2(OH)_2]^{4-}$ dimer represents a stable intermediate in the pathway to the assembly of $[Fe_2(\mathbf{1})_3]^{6-}$. The displacement of the bridging hydroxides of $[Fe_2(\mathbf{1})_2(OH)_2]^{4-}$ by an additional ligand to generate the dinuclear triple helix can readily be detected by visible spectroscopy. An isosbestic point at λ = 584 nm observed during incremental addition of a solution of $\mathbf{1}$ to an aqueous solution (pH = 9)[31] of $[Fe_2(\mathbf{1})_2(OH)_2]^{4-}$ indicates that the specific type of complex present in water is under stoichiometric control of the following reaction:

$$[Fe_2(\mathbf{1})_2(OH)_2]^{4-} + \mathbf{1}\text{-}H_2^{2-} \rightleftharpoons [Fe_2(\mathbf{1})_3]^{6-} + 2H_2O$$
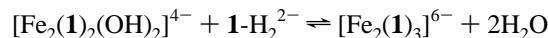
Visible spectroscopic studies with a naturally-occurring bis-(catecholamide) siderophore[4] also reveal a dependence on stoichiometry. Solutions comprised 1:1 of this siderophore and iron exhibit complex spectra unlike $[Fe_2(\mathbf{1})_2(OH)_2]^{4-}$, suggesting that this siderophore does not form a bridged dimer. However, solutions composed of a 2:3 ratio display a λ_{max} at 488 nm ($[Fe_2(\mathbf{1})_3]^{6-}$ λ_{max} = 488 nm). The similarity between the solution behavior of the bis(catecholamide) siderophore and $\mathbf{1}$ under 2:3 stoichiometry suggests a dinuclear triple helix could be involved in bacterial sequestration of iron. Recognition of the latter as the sequestered form is readily envisioned to occur at a cell surface receptor similar to that of ferric enterobactin, the best known tris(catecholamide) siderophore. The cell receptor recognizes ferric enterobactin by the ferric tris-(catecholamide) portion of the complex rather than the triserine organic fragment anchoring the catecholamides.[32] A ferric dinuclear triple helix composed of bis(catecholamide) ligands has two potential tris(catecholamide) recognition sites. Bacterial recognition of a single end of a dinuclear triple helix may constitute a unifying theme among the structurally diverse bis-(catecholamide) siderophores.

Supporting Information Available: Text presenting crystal data and X-ray experimental details for $(Et_4N)_6[Fe_2(\mathbf{1})_3]$ and $(Me_4N)_4[Fe_2$-$(\mathbf{1})_2(OH)_2]$ along with a visible titration between these two species, a table giving a spectroscopic chararacterization of $\mathbf{1}$-H_4, and diagrams of the bis(catecholamide) and $\mathbf{1}$-H_4 (5 pages). Ordering information is given on any current masthead page.

(25) Crystal data for $(Me_4N)_4[Fe_2(\mathbf{1})_2(OH)_2]\cdot8.5H_2O$: red plates in monoclinic $C2/c$ (No. 15), a = 18.462(2) Å, b = 13.990(2) Å, c = 23.252-(2) Å, β = 94.47(1)°, V = 5987(1) Å^3, Z = 4; 3898 unique reflections collected (203 K, 5° < 2θ < 46°); 2382 reflections ($|F_o|$ > 3$\sigma(F_o)$, 5° < 2θ < 46°), R (R_w) = 5.7(7.3).
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