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Crystal and Molecular Structures of Ionophore−Siderophore Host−Guest Supramolecular Assemblies Relevant to Molecular Recognition

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Abstract: Ionophore-siderophore host−guest assemblies composed of 18-crown-6 and ferrioxamine B, benzo-18-crown-6 and ferrioxamine B, and *cis-syn-cis*-dicyclohexano-18-crown-6 and ferrioxamine B were successfully crystallized, and their structures were determined by single-crystal X-ray diffraction. All three crystal lattices also include solvated Mg(II) and perchlorate ions. The ionophore−siderophore host−guest assembly is noncovalently held together by a hydrogen bonding interaction between the pendant protonated amine in the second coordination sphere of ferrioxamine B and the hydrogen bond acceptor oxygen atoms in the crown ether. The crystals of 18-crown-6:ferrioxamine B host−guest assembly are monoclinic, with space group $P2_1/c$, and four molecules per unit cell with dimensions $a = 19.8327(11)$ Å, $b = 20.4111(11)$ Å, $c = 15.1698(8)$ Å, and $\beta = 96.435(1)°$. The crystals of benzo-18-crown-6:ferrioxamine B host−guest assembly are triclinic, with space group $P\bar{1}$, and two molecules per unit cell with dimensions $a = 11.1747(10)$ Å, $b = 16.0580(15)$ Å, $c = 18.4175(17)$ Å, $\alpha = 80.469(3)°$, $\beta = 81.481(3)°$ and $\gamma = 70.212(2)°$. The crystals of *cis-syn-cis*-dicyclohexano-18-crown-6:ferrioxamine B host−guest assembly are monoclinic, with space group $P2_1/c$, and four molecules per unit cell with dimensions $a = 20.1473(13)$ Å, $b = 21.5778(15)$ Å, $c = 14.8013(10)$ Å, and $\beta = 94.586(2)°$. The crystal structures of all three host−guest assemblies contain a racemic mixture of Λ-N-*cis*, *cis* and Δ-N-*cis, cis* coordination isomers of ferrioxamine B. The crystal structures indicate that the steric rigidity of the benzo-18-crown-6 and *cis-syn-cis*-dicyclohexano-18-crown-6 cavity has a pronounced effect on the conformation of the crown ring and ultimately on the hydrogen bonding interactions between the crown ethers and ferrioxamine B. The structural parameters and the conformational features of the ferrioxamine B guests compare very well with each other and with those of the ferrioxamine B structure obtained in the absence of a host. Structural features relevant to siderophore molecular recognition are discussed.

Introduction

Microbial iron acquisition is a very sophisticated natural process incorporating interesting and complex chemical problems.[1] This elaborate scheme involves sequestering Fe(III) from the environment by chelating agents, molecular recognition of an iron-chelate complex at the cell membrane, transport across the cell membrane, and iron deposition at an appropriate site within the cell.[2−5] The need for such a complex system arises from the indispensable role of iron in many biological processes, combined with the very low solubility of iron at pH = 7.4 in aqueous aerobic conditions ($[Fe_{aq}^{3+}]_{tot} = 10^{-10}$ M).[5] Microorganisms produce siderophores, strong Fe(III)-specific chelators, to effectively solubilize and transport iron. The microbial bioavailability of iron is largely determined by the Fe(III) chelating affinity of these siderophores.[2−8]

An essential step in microbial iron acquisition involves molecular recognition of the siderophore−iron complex at the cell membrane and subsequent transport across the membrane into the cell, or release of iron to a membrane bound carrier. The siderophore-iron complexes are recognized at the cell surface by high-affinity siderophore receptors and enter the cell through outer membrane proteins in an energy dependent process.[1,9−14] Recent crystallographic data for a siderophore

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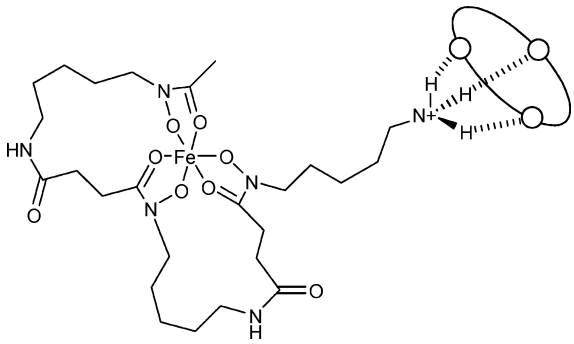
bound to a siderophore-receptor demonstrate that both the first and the second coordination shells of Fe(III) are involved in hydrogen bonding interactions with the receptor during the recognition process.[15−17] The interaction between siderophore-iron complex and periplasmic binding protein also displays similar first and second coordination shell recognition.[18] The outer membrane receptor for the ferrioxamines, FoxA, has been cloned and sequenced.[19,20] The structure−function analysis between FoxA and the ferrioxamines also indicates that receptor interaction with the first coordination shell as well as the siderophore backbone are essential for receptor recognition.[19] Recently, we have modeled ferrioxamine B molecular recognition at the interface between the cell and the environment using second coordination shell host−guest complexation.[21−32] Such second coordination shell recognition involves supramolecular host−guest assembly formation,[33] where a low molecular weight host capable of recognizing a siderophore guest is used to mimic aspects of a high molecular weight membrane-bound receptor protein. Such a simple model system involving a supramolecular host−guest assembly does not fully duplicate recognition found in a natural receptor, but provides the means for a systematic and controlled examination of various chemical and structural factors affecting the recognition and the transport process.

Desferrioxamine B (Scheme 1), a trihydroxamate siderophore produced by actinomycetes, is well studied.[8] Structural studies of ferrioxamine B are of great importance as this siderophore is of considerable chemical and pharmaceutical interest. In siderophore-mediated iron transport, the structural properties of ferrioxamine B, both first and second coordination shell, are believed to play an important role in the recognition and Fe(III) uptake mechanism.[34] The presence of the pendant protonated

Scheme 1. Ferrioxamine B/Ionophore Host-Guest Assembly



amine in the second coordination shell of ferrioxamine B can potentially provide the ferrioxamine B system with additional hydrogen bonding capabilities during the receptor recognition process. The pharmaceutical use of desferrioxamine B (Desferal) in the treatment of acute iron poisoning and in chronic iron overload resulting from transfusion therapy for β-thalassemia (Cooley's anemia)[35−38] underlines the importance of structural information on ferrioxamine B and its interactions with other molecules. Along with the thermodynamic and kinetic properties of ferrioxamine B, structural information for ferrioxamine B and its interactions with other molecules may aid in designing a more efficacious drug for the treatment of iron toxicity without adverse side effects.

Synthetic crown ethers and some natural antibiotics are known to recognize hydrophilic cations via host−guest interactions and facilitate their transfer from aqueous phases to low polarity lipophilic phases.[39−43] Past investigations have shown that a lipophilic crown ether is capable of recognizing the terminal protonated amine group of ferrioxamine B ($FeHDFB^+$, Scheme 1), through a second coordination shell host−guest interaction, and promote its extraction from water into a more lipophilic chloroform phase.[21−28,31,32] This second coordination shell recognition has been used to investigate and understand factors affecting bulk liquid membrane (BLM) transport of ferrioxamine B by several ionophore hosts.[29,30] The host−guest association constant (K_{h-g}), their transport across different phases (flux), and extraction constants (K_{ex}), have been shown to be sensitive to host cavity size, dimensionality, stereochemistry, solvation shell, and counteranions.

Understanding the details of these supramolecular assemblies and their movement across a multiphase solvent system is relevant to a number of applications. Use of siderophores and their analogues for the treatment of iron-overload diseases and metal toxicity is well established.[2,35−38,44] Ionophore mediated

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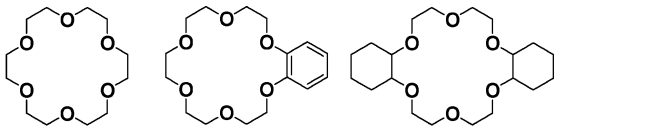
Scheme 2. Neutral Crown Ethers



18-crown-6 **18C6** | benzo-18-crown-6 **B18C6** | *cis-syn-cis*-dicyclohexano-18-crown-6 **DC18C6**

extraction can potentially be an excellent tool for bioremediation, phase-transfer catalysis, trace metal recovery, liquid membrane transport, and separation of industrial and biological materials. In our attempt to study the structures of ionophore−siderophore assemblies by single-crystal X-ray diffraction, we have successfully determined three crystal structures of ionophore−siderophore host−guest complexes involving three different crown ethers (Scheme 2), and ferrioxamine B ($FeHDFB^+$): 18-crown-6:$FeHDFB^+$, benzo-18-crown-6:$FeHDFB^+$ and *cis-syn-cis*-dicyclohexano-18-crown-6:$FeHDFB^+$. Here, we report these crystal structures along with a comparative analysis of the first and second coordination shell of ferrioxamine B in the presence and absence of an ionophore host in the context of molecular recognition and Fe(III) transport efficiency.

Experimental Section

Materials and Crystallization Methods. All solutions were prepared in deionized water. All pH measurements were made using a Corning 250 pH/ion meter equipped with an Orion ROSS pH electrode filled with 3.0 M NaCl solution. The pH was adjusted with NaOH or $HClO_4$ as needed. Stock solutions of 1.0 M $Mg(ClO_4)_2$ were prepared from solid magnesium perchlorate hydrate (Aldrich 99+%) and standardized by passing through a Dowex 50 W−X8 strong acid cation-exchange column in H^+ form. The 2.0 M $HClO_4$ stock solution was prepared from concentrated perchloric acid (Fisher 70%) and standardized by titration with standard NaOH solution to the phenolphthalein end point. Iron(III) perchlorate stock solution (0.1 M) was prepared at pH 1.0 from recrystallized $Fe(ClO_4)_3$ (Aldrich), and standardized spectrophotometrically in strong acid,[45] and titrimetrically by reduction with Sn(II) and titrated with the primary standard potassium dichromate.[46] Carbonate free NaOH (0.1 M) was prepared by diluting Fisher 1.0 M NaOH with deionized water purged with argon for 45 min, standardized by titration with standard 0.2 M HCl (Fisher) to the phenolphthalein end point. A desferrioxamine B, H_4DFB^+ (50 mM), solution was prepared in 0.1 M $Mg(ClO_4)_2$. The ferrioxamine B complex was formed by adding 1 equiv of $Fe(ClO_4)_3$ stock solution to the desferrioxamine B solution and increasing the pH to 4.4 by the slow addition of 1.0 M $Mg(OH)_2$. The aqueous solution was allowed to evaporate to obtain a viscous dark red semisolid mixture of ferrioxamine B complex, $(FeHDFB^+)ClO_4$, and $Mg(ClO_4)_2$, which was then dissolved in a 1:1 ethanol:methanol solution. (*CAUTION: care must be taken while handling perchlorate salts with organic ligands as they are potentially dangerous when dried.*) The host−guest complexes of the crown ethers (18-crown-6, benzo-18-crown-6 and *cis-syn-cis*-dicyclohexano-18-crown-6) with ferrioxamine B were formed by adding 1.2 equiv of crown ether to the ferrioxamine B solution in ethanol/methanol. Thin red plates of 18-crown-6:ferrioxamine B, benzo-18-crown-6:ferrioxamine B and *cis-syn-cis*-dicyclohexano-18-crown-6:ferrioxamine B were grown by very slow evaporation.

Crystal Data. All X-ray diffraction data sets were collected using a Bruker SMART diffractometer in the omega scan mode. The frame integration and the unit-cell refinement were carried out using SAINT. The empirical absorption correction was carried out using SADABS and all the calculations were performed using the NRCVAX suite of programs[47] with scattering factors taken from published data.[48] The crystal data and the summary of data collection and structure refinement are summarized in Table 1. The positions of the hydrogen atoms on the protonated amine were located in a difference map, whereas the positions of the remaining hydrogen atoms were calculated. Crystallographic data (without structure factors) for all three structures reported here have been deposited with the Cambridge Crystallographic Data Center as a supplementary publication, with identification numbers.

Results and Discussion

General Description. The crystal structures show a host−guest interaction between the crown ether ionophore and the ferrioxamine B siderophore that results from hydrogen bonding between the oxygen atoms on the crown ether ring and the hydrogen atoms on the protonated amine in the second coordination sphere of ferrioxamine B. Perspective single-molecule drawings of 18-crown-6:ferrioxamine B (18C6:$FeHDFB^+$), benzo-18-crown-6:ferrioxamine B (B18C6:$FeHDFB^+$) and *cis-syn-cis*-dicyclohexano-18-crown-6:ferrioxamine B (DC18C6:$FeHDFB^+$) host−guest assemblies as determined by X-ray diffraction are shown in Figure 1, along with the appropriate atom numbering schemes. The crystal structures clearly reveal the formation of host−guest complexes of 1:1 stoichiometry in the second coordination shell of ferrioxamine B. Although all three host−guest complexes form very similar 1:1 complexes, they significantly vary in their hydrogen bonding interactions, which will be discussed later in the context of crown ether conformation and electronic properties.

The unit cells of all three crystal structures contain positively charged ferrioxamine B and solvated Mg(II), negatively charged perchlorate, neutral crown ether, and water and ethanol solvent. All three structures have a perchlorate anion on the same side of the crown ether as the pendant protonated amine at a distance of approximately 5 Å. Other perchlorate ions are adjacent to the Mg(II) centers, which are present as hexaaquomagnesium(II) perchlorate in the crystal lattice of 18-crown-6:ferrioxamine B and benzo-18-crown-6:ferrioxamine B, and as ethanolpentaaquamagnesium(II) perchlorate in the crystal lattice of *cis-syn-cis*-dicyclohexano-18-crown-6:ferrioxamine B host−guest assemblies. There is significant noncovalent interaction between the water molecules coordinated to the Mg(II) and perchlorate anions, as well as the backbone carbonyl groups on the ferrioxamine B molecule. The Mg(II) is believed to act as a dehydrating agent during the course of crystallization, as it removes the ferrioxamine B second-sphere H_2O molecules as well as H_2O that may be hydrogen bonded to the crown ether cavity.[34]

Crown Ether Hosts. The unsubstituted host 18-crown-6 is present in a pseudo D_{3d} symmetrical conformation. The six oxygen atoms are alternatively placed above and below their mean plane (Figure 2a). The Newman projections seen through C−C bonds show an almost ideal gauche conformation of the oxygen atoms in the macrocycle ring. The C−C and C−O bond distances, as well as the dihedral angles, match well with those seen in numerous other cation:18-crown-6 complexes.[49,50] The

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Table 1. Crystal Data and Structure Refinement for Host−Guest Complexes of Ferrioxamine B ($FeHDFB^+$) with 18-crown-6, Benzo-18-crown-6 and *cis-syn-cis*-Dicyclohexano-18-crown-6

	18-crown-6:$FeHDFB^+$	benzo-18-crown-6:$FeHDFB^+$	*cis-syn-cis*-dicyclohexano-18-crown-6:$FeHDFB^+$
compound	$FeMg_{0.50}Cl_2C_{38}H_{85}N_6O_{28.50}$	$FeMg_{0.50}Cl_2C_{41}H_{82}N_6O_{29.50}$	$FeMg_{0.50}C_{l2}C_{46.67}H_{84.50}N_6O_{26.50}$[a]
formula weight	1221.01	1270.02	1292.64
temperature, K	173	173	173
crystal dimensions, mm	0.35 × 0.20 × 0.01	0.25 × 0.25 × 0.20	0.20 × 0.20 × 0.20
crystal system	monoclinic	triclinic	monoclinic
space group	$P2_1/c$	$P\bar{1}$	$P2_1/c$
cell constants			
a, Å	19.8327(11)	11.1747(10)	20.1473(13)
b, Å	20.4111(11)	16.0580(15)	21.5778(15)
c, Å	15.1698(8)	18.4175(17)	14.8013(10)
α, deg	N/A	80.469(3)	N/A
β, deg	96.435(1)	81.481(3)	94.586(2)
γ, deg	N/A	70.212(2)	N/A
cell volume, Å^3	6102.2(6)	3051.7(5)	6414.0(7)
Z	4	2	4
D_{calc}, g cm^{-3}	1.329	1.382	1.339
μ_{calc}, cm^{-1}	4.2	4.3	4.0
2*θ* range, deg	5.00 < 2*θ* < 50.00	5.00 < 2*θ* < 50.00	5.00 < 2*θ* < 50.00
range of *h,k,l*	−23 to 23, 0 to 24, 0 to 17	−11 to 12, 0 to 17, −19 to 19	−23 to 23, 0 to 25, 0 to 17
no. of reflection collected	22463	22334	24793
no. of independent reflections	10532	8025	10952
no. of reflection with $I_{net} > 2.5\sigma(I_{net})$	5643 [*R*(int) = 0.038]	4537 [*R*(int) = 0.068]	4497[*R*(int) = 0.055]
	$R_f = 0.071$, $R_w = 0.086$	$R_f = 0.070$, $R_w = 0.082$	$R_f = 0.085$, $R_w = 0.096$
	GOF = 2.4788	GOF = 1.6680	GOF = 2.4128
R indices (all data)	$R_f = 0.125$, $R_w = 0.091$	$R_f = 0.127$, $R_w = 0.105$	$R_f = 0.188$, $R_w = 0.114$
largest diffraction peak and hole, eÅ^{-3}	0.630 and −0.380	1.060 and −0.530	1.140 and −0.650

[a] The empirical formula is inaccurate because of unassignable hydrogens and disorder about Mg(1). The dataset is extremely weak, hence it is not possible to assign the hydrogen atoms on the free water molecules. The oxygen atoms O(91) and O(92) are noncoordinated solvent molecules of which O(91) only has approximately 50% occupancy. Mg(1) appears to be surrounded by 5 water molecules and one ethanol which is disordered with the water trans to it. The oxygen atom O(73) is the overlapped water and ethanol −OH, C(74) is part of the ethanol and C(75) is an overlap of the ethanol $-CH_3$ and a water molecule that is likely hydrogen bonded to the water part of O(73). One of the perchlorate anions is disordered and is modeled by Cl(2) and two tetrahedral oxygens: O(85) to O(88) and O(85a) to O(88a).

symmetrical ring conformation and distribution of oxygen atoms in the macrocycle make the 18-crown-6 cavity ideal for guest complexation.

The benzo-18-crown-6 macrocycle has significant steric rigidity and bulk imposed by the benzo group, which significantly alters the positions of the oxygen atoms compared to the unsubstituted 18-crown-6 (Figure 2b). The conjugated benzo ring prevents C−C bond rotation, which imparts rigidity to the ring and forces coplanarity on the two adjacent oxygen atoms. The horizontal side view of the benzo-18-crown-6 actually shows three adjacent oxygen atoms closest to the benzo group on one side of the crown cavity mean plane while the remaining three oxygen atoms are on the opposite side (Figure 2b, row 2). This displacement of the oxygen atoms, due to the lack of C−C bond rotation, is also seen in the Newman projection through this bond (Figure 2b, rows 3 and 4). The overall shape of the benzo-18-crown-6 is not flat like 18-crown-6, but slightly curved giving rise to a distinct concave face that contains the benzo group.

Although the host−guest assembly of *cis-syn-cis*-dicyclohexano-18-crown-6 and ferrioxamine B was crystallized from a solution containing a mixture of both *cis-syn-cis* and *cis-anti-cis* isomers, the crystals isolated exclusively contain the *cis-syn-cis* isomer. The two cyclohexyl rings add significant steric rigidity to the 18-crown-6 macrocycle, which is reflected by the positions of the ring oxygen atoms. Figure 2(c) clearly shows that the oxygen atoms are no longer alternating above and below the mean plane as seen with unsubstituted 18-crown-6, but rather are alternating in pairs. The four oxygen atoms next to the two cyclohexyl rings are on one side of the crown cavity mean plane, while the remaining two oxygen atoms are on the opposite side (Figure 2c, row 2). The Newman projections show only a slight variation of the position of the oxygen atoms compared to that of the 18-crown-6 macrocycle, indicating similar free rotation about the C−C bond. The overall shape of the *cis-syn-cis*-dicyclohexano-18-crown-6 is a "bowl like" conformation. Four oxygen atoms that are nearest to the two cyclohexyl groups are pointing toward the inside of the bowl, while the remaining two oxygen atoms are pointing away from the bowl.

Ferrioxamine B Guest. Although chelation of Fe(III) by the linear desferrioxamine B siderophore can hypothetically give rise to 16 different geometrical and optical isomers of ferrioxamine B,[51] the solid-state structures of ferrioxamine B in all three host−guest assemblies display an isomeric selectivity. The crystal structures of all three host−guest assemblies contain only a racemic mixture of Δ-N-*cis,cis* and Λ-N-*cis,cis*-ferrioxamine B isomers, as observed in the crystal structures of ferrioxamine B,[34] D_1,[52] and E.[53] The asymmetry of the hydroxamate moiety gives rise to an uneven charge distribution at the oxygen atoms coordinated to the Fe(III). The Fe(III)-oxime O bond distances are systematically shorter than the Fe(III)-carbonyl O bond distances, consistent with the localization of a greater negative charge on the oxime oxygen compared to the carbonyl oxygen atoms. The *cis(fac)* configuration about the Fe(III) center results

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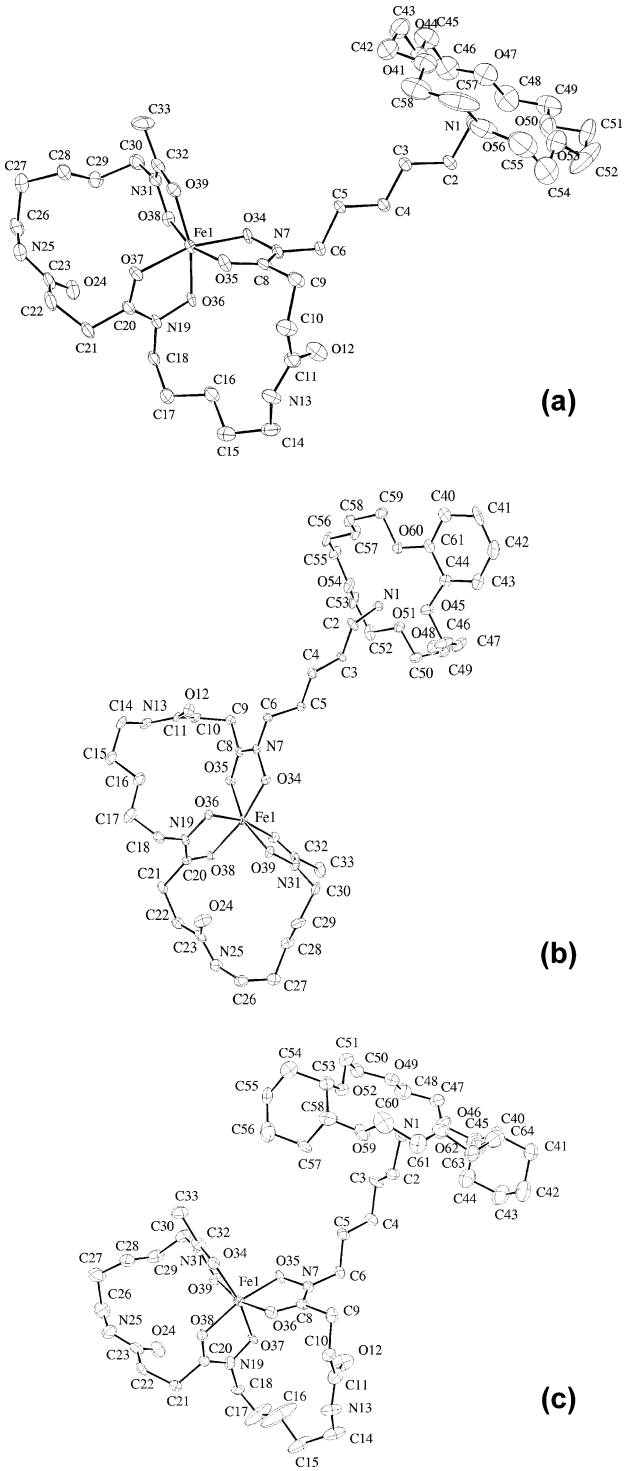

Figure 1. Perspective single-molecule drawings of (a) 18-crown-6:ferrioxamine B (b) benzo-18-crown-6:ferrioxamine B (c) *cis-syn-cis*-dicyclohexano-18-crown-6:ferrioxamine B host−guest assembly based on the crystal structures, showing atom numbering

in two distinct trigonal octahedral faces with respect to the pseudo 3-fold axis, an oxime face and a carbonyl face. The geometry at the Fe(III) center deviates from perfect octahedral primarily due to the uneven oxygen atom charge distribution and the chelate bite angle. All three ferrioxamine B guest structures and ferrioxamine B itself[34] display this deviation as indicated by the O−Fe−O bond angles, normalized bite and

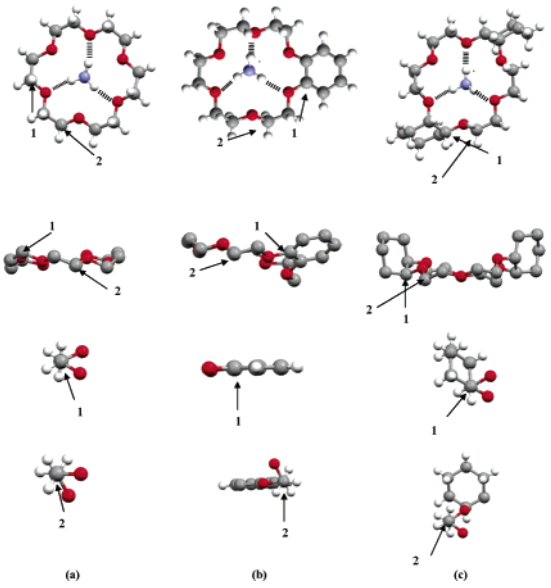

Figure 2. Ball-and-stick structures of (a) 18-crown-6 (b) benzo-18-crown-6 and (c) *cis-syn-cis*-dicyclohexano-18-crown-6 host structures based on the crystal structures of their host−guest complexes with ferrioxamine B. Row 1, top view of the crown cavity with the pendant protonated amine. Three shortest N−H···O distances: 2.884, 2.866, and 2.871 Å for 18C6:FeHDFB$^+$, 2.804, 2.808, and 2.920 Å for B18C6:FeHDFB$^+$, and 2.840, 2.868, and 2.942 Å for DC18C6:FeHDFB$^+$. Row 2, side view. Rows 3 and 4, Newman projections. Arbitrary labels of *1* and *2* are given to two C atoms for clarity in comparing different perspective views of each structure.

the trigonal twist angle. For comparison, the O−Fe−O bond angles of 90°, trigonal twist angles of 60° and a bite of 1.414 give the minimum energy configuration for idealized octahedral coordination, which can be exhibited by the spherically symmetrical high-spin d^5 Fe(III) ion.[54,55] These parameters deviate significantly from ideality for the ferrioxamine B structures and together are indicative of the first coordination shell distortion caused by the constraints of the five-membered chelate rings, the extent of steric interaction between the chelating rings, and the separation between the Fe(III) center and the two trigonal faces formed by the oxime oxygen and carbonyl oxygen atoms. The structural parameters for the first coordination shell of the ferrioxamine B molecule in all three host−guest assemblies and in the absence of a host molecule are listed in Table 2.

The parameters in Table 2 illustrate insignificant differences in the inner coordination shell between ferrioxamine B and the three host−guest assemblies reported here. The overall high level of congruence is illustrated in Figure 3. All three supramolecular assemblies have the pendant pentylammonium chain pointing away from the amide connecting rings and toward the carbonyl face of the first coordination shell octahedron. Ferrioxamine B in the absence of second-sphere host−guest complexation has flexibility in orienting its protonated amine side chain. This flexibility is clearly shown by the out and upward orientation of the protonated amine in the absence of an ionophore host. The superimposed structures of these four ferrioxamine B molecules also display a slight variation in one of the two amide connecting rings (Figure 3). This conformational difference is present only in the ferrioxamine B structure obtained in the absence of a host. The conformational differ-

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Table 2. Structural Parameters for the First Coordination Shell of Ferrioxamine B and Various Ferrioxamine B-Ionophore Assemblies

parameter[a]	ferrioxamine B[b]	18C6:ferrioxamine B	B18C6:ferrioxamine B	DC18C6:ferrioxamine B
Fe—O(C) (Å)	2.037	2.027(4)	2.043(26)	2.036(7)
Fe—O(N) (Å)	1.981	1.984(10)	1.979(16)	1.987(2)
C—O (Å)	1.282	1.272(1)	1.282(9)	1.278(21)
N—O (Å)	1.377	1.374(7)	1.377(10)	1.386(24)
N—C (Å)	1.317	1.313(13)	1.306(10)	1.308(11)
normalized bite[c]	1.27	1.26	1.26	1.26
trigonal twist angle (deg)	41.2	41.8	42.0	43.6
O—Fe—O (deg)	78.7	78.4(5)	78.1(9)	78.3(3)
O—Fe—O axial (deg)	164.23	164.7(2)	164.3(33)	166.0(15)
log K_{h-g}[d]		3.52	2.82	3.76
ΔH_{h-g} (kJ mol^{-1})[d]		−59.4	−28.2	−37.4

[a] Average values. The values in the parentheses indicate the standard deviation. [b] Ref 34. [c] Normalized bite: $2\sin(\theta/2)$, θ = O—Fe—O angle. [d] Ref 32.

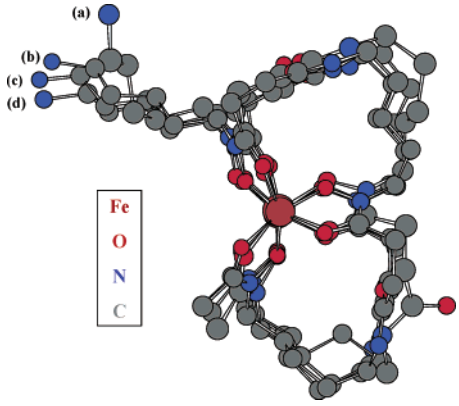

Figure 3. Superimposed ball-and-stick structures of four ferrioxamine B molecules from different assemblies. (a) ferrioxamine B alone,[34] (b) benzo-18-crown-6:ferrioxamine B, (c) 18-crown-6:ferrioxamine B and (d) *cis-syn-cis*-dicyclohexano-18-crown-6:ferrioxamine B. Crystallographic data for ferrioxamine B was obtained from Cambridge Structural Database.

ences, however, are minimal and are most likely the result of differences in packing forces in the crystal lattices.

Host−Guest Interactions. The oxygen atoms in the hypothetical ideal crown ether ring are displaced alternately above and below the median plane of the ring. Such an arrangement results in two approximately parallel, and nearly equilateral, triangles, rotated 60° to one another. The absence of a substituent group makes all oxygen atoms within the 18-crown-6 macrocycle electronically equivalent and equally basic. The ferrioxamine B guest can "perch" above the mean plane of the oxygen atoms in this idealized crown ether ring. This is the case for 18-crown-6. The structural rigidity of benzo-18-crown-6 and *cis-syn-cis*-dicyclohexano-18-crown-6 changes the ring conformation and significantly alters the hydrogen bonding interactions between the three crown ethers and the ferrioxamine B molecule. In addition, the presence of a benzo substituent considerably decreases the basicity of the oxygen atoms.

The lack of structural rigidity of 18-crown-6 gives rise to an almost ideal alignment of the oxygen atoms in the crown ether ring. Three hydrogen-bonding interactions hold the pendant protonated amine of the guest ferrioxamine B molecule at the center of the crown ether cavity (Figure 2a, row 1) in a "perching" conformation ca. 0.75 Å from the least-squares plane of the oxygen atoms. Hydrogen bonding interactions between the protonated amine and alternate oxygen atoms in the crown ether is indicated by the short N−H···O distances of 2.844, 2.866, and 2.871 Å. These distances are in good agreement with hydrogen bond distances in various ammonium and alkylammonium complexes with crown ethers.[56−62] The N−H···O angles (nitrogen−hydrogen−oxygen) of 167.1°, 164.05°, and 168.81° are internally consistent and fall in the range of N−H···O angles reported for other alkylammonium complexes.[41,49,50,63−67] The conformation of the crown ether forces the remaining oxygen atoms to be oriented away from the protonated amine; as a result, these oxygen atoms display unfavorable distances and angles for hydrogen bonding.

The rigidity of the benzo group makes the "perching" conformation in the assembly B18C6:FeHDFB$^+$ slightly less defined; however, the nitrogen atom of the pendant protonated amine is displaced from the least-squares plane of the oxygen atoms in benzo-18-crown-6 by ca. 0.60 Å. The structural rigidity of the crown ether is further illustrated by the fact that the O atom hydrogen bond acceptors are no longer alternating up and down relative to the least-squares plane (Figure 2b, row 3). Consequently, hydrogen bonding interactions between the protonated amine side chain and the crown ether ring are no longer readily defined as involving alternate oxygen atoms. In addition, the pendant protonated amine is not centrally located at the benzo-18-crown-6 cavity, but is docked away from the less basic oxygen atoms adjacent to the benzo group (Figure 2(b), row 1). This phenomenon is clearly illustrated by the spread in the N−H···O distances, which for the three alternating oxygen atoms (O48, O54, and O60) are 2.804, 2.808, and 2.920 Å, respectively. However, oxygen atom O45, which is between oxygen atoms O48 and O60, is 2.839 Å from the protonated amine nitrogen atom. The N−H···O angles for the three alternating oxygen atoms are 174.05°, 174.02°, and 164.93°. These angles and the N−H···O distances are consistent with the angles and the distances observed for other alkylammonium complexes with benzo-18-crown-6.[41,59,63−67]

Cis-syn-cis-dicyclohexano-18-crown-6 is hydrogen bonded to the pendant protonated amine of ferrioxamine B via the inside

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of its "bowl-like" structure, placing the ferrioxamine B molecule and the two cyclohexyl groups on the same face of the crown ether. A preference of the guest for the sterically crowded face of the host seen above with benzo-18-crown-6 and here for the *cis-syn-cis*-dicyclohexano-18-crown-6, is unexpected. However, the alignment of more oxygen atoms along the sterically crowded face of the crown ethers explains the observed preference. The ordered bowl conformation of the *cis-syn-cis*-dicyclohexano-18-crown-6 significantly reduces the "perching" conformation of the host−guest assembly, which is demonstrated by the displacement of the nitrogen atom of the protonated amine from the least-squares plane of the oxygen atoms by ca. 0.60 Å. The pendant protonated amine is centrally placed in the crown cavity despite the steric rigidity. This suggests that the electronic properties of the oxygen atoms present in the *cis-syn-cis*-dicyclohexano-18-crown-6 macrocycle are very similar. The hydrogen atoms on the protonated amine of ferrioxamine B are aligned with the alternate oxygen atoms in the crown ether and display short N−H···O distances of 2.840, 2.868, and 2.942 Å and N−H···O angles of 171.72°, 162.08°, and 166.9°. These distances and angles are in good agreement with various ammonium and alkylammonium complexes with crown ethers.[41,59,63−67] In addition to the alternating oxygen atoms present on one face of the crown ring, two other oxygen atoms on the opposite face are also at a distance 2.809 and 2.981 Å from the nitrogen atom of the protonated amine. Although these oxygen atoms are not aligned in the direction of the hydrogen bonding hydrogen atoms, their close proximity suggests they may contribute to the stability of the host−guest complex.

Implications for Molecular Recognition in Solution. Previous studies on ionophore−siderophore host−guest assemblies have been in solution.[21−32,68] These solution studies investigated the thermodynamics of siderophore guest recognition by the host, the extractability of the host−guest assembly into a lipophilic phase, and host carrier mediated flux of the guest across a lipophilic membrane. The results clearly indicate that the host−guest interaction and other physical properties of the ionophore-siderophore host−guest assemblies are strongly influenced by a series of parameters: host cavity size, dimensionality, stereochemistry, solvation shell, and counteranions. In an attempt to better understand the factors affecting host−guest complexation and siderophore molecular recognition, here we analyze the conformation and other geometrical features of the host and the guest molecules, and their interactions with each other. The crystallographic data presented here are discussed with full acknowledgment of the limitations in extrapolating solid-state structural information to dynamic solution behavior.

Although a racemic mixture of only the Δ-N-*cis,cis* and Λ-N-*cis,cis*-ferrioxamine B isomers are observed in the crystal structures of ferrioxamine B, the Al(III), Ga(III), and Cr(III) complexes of desferrioxamine B exhibit both trans and cis isomers in solution.[69,70] The coordination chemistry of Fe(III) is similar to that of Al(III), Cr(III), and Ga(III) (Ga(III) and Fe(III) have equivalent ionic radii), and thus, the Fe(III) complex of desferrioxamine B should be able to interconvert between a *cis* and *trans* configuration. The exclusive presence of only two *cis* isomers in four different crystal structures of ferrioxamine B suggests that the Δ-N-*cis,cis* and Λ-N-*cis,cis* isomers are likely the lowest energy isomeric configuration for Fe(III) complexes of desferrioxamine B.

Solution studies on host−guest complex formation between crown ethers and ferrioxamine B show significant associative interaction with host−guest association constants (K_{h-g}) in wet $CHCl_3$ on the order of 10^3-10^5 M^{-1}.[23,32] Among the three host−guest assemblies for which we report structural data, the ΔH_{h-g} values for host−guest complexation in $CHCl_3$ vary over the range −28 to −59 kJ/mol, consistent with the host−guest interaction being due to hydrogen bond formation. However, no correlation is observed between the N−H···O distance (average of three hydrogen bond distances) in the solid state and either the K_{h-g} or ΔH_{h-g} associated with host−guest association in solution (Table 2). This may be due to the limited number of data points. However, an isothermal relationship between ΔH_{h-g} and ΔS_{h-g} suggests that variations in K_{h-g} at 298 K are due to entropic effects.[32] These considerations suggest that the thermodynamics in solution are significantly influenced by solvent−solute interactions and are not entirely similar to the crystallographic data where the thermodynamics of host−guest complexation are also influenced by the crystal packing forces.

The strength of the host−guest assembly may depend to a degree on the position of the pendant protonated amine within the crown cavity, as illustrated in Figure 2, row 1. The most symmetric association of the pendant amine guest with the host (18C6:$FeHDFB^+$) produces the most exothermic solution complexation reaction (ΔH_{h-g}) and most stable assembly (K_{h-g}), whereas the least symmetric association reported here (B18C6:$FeHDFB^+$) produces the least exothermic solution reaction and least stable assembly (Table 2). The symmetry of the DC18C6:$FeHDFB^+$ assembly is consistent with its intermediate solution stability and ΔH_{h-g}.

Summary and Conclusions

Three crystal structures reported here of host−guest complexes involving three different crown ethers and ferrioxamine B (18-crown-6:$FeHDFB^+$, benzo-18-crown-6:$FeHDFB^+$, and *cis-syn-cis*-dicyclohexano-18-crown-6:$FeHDFB^+$) represent the first reported structures of ionophore-siderophore host−guest assemblies. The structures confirm the ability of the crown ether cavity to recognize ferrioxamine B through second coordination sphere host−guest complexation with 1:1 stoichiometry. The crystal structures indicate that the steric rigidity of the benzo-18-crown-6 and *cis-syn-cis*-dicyclohexano-18-crown-6 has a pronounced affect on the conformation of the crown ether, that in turn affects the hydrogen bonding interaction between the crown ethers and ferrioxamine B. The structural parameters for the ferrioxamine B molecule in all three crystal structures show a high degree of congruence and exclusively contain Δ-N-*cis,cis* and Λ-N-*cis,cis* isomers. This isomeric selectivity from the 16 possible isomers suggests that the Δ-N-*cis,cis* and Λ-N-*cis,cis* isomers are possibly the lowest energy isomeric forms. The presence of the pendant protonated amine in the second coordination shell of ferrioxamine B is certainly capable of

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providing the ferrioxamine B complex with additional hydrogen bonding opportunities during the cell receptor recognition, and other smaller host molecule recognition processes. The orientation of the pendant protonated amine toward the more open carbonyl trigonal face of the first coordination shell of ferrioxamine B in these assemblies may also be of significance in the cell receptor recognition process, as the oxygen atoms on this face are available for intermolecular hydrogen bonding as illustrated by the crystal structure of ferrichrome with FhuA.[15]

Acknowledgment. We thank the National Science Foundation (CHE-0079066) for financial support.

Supporting Information Available: Crystallographic data in CIF format for each of the three structures reported here (also deposited with the Cambridge Crystallographic Data Centre). This material is available free of charge via the Internet at http://pubs.acs.org.

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