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Molecular recognition of stable metal complexes through second-sphere coordination by macrocycles

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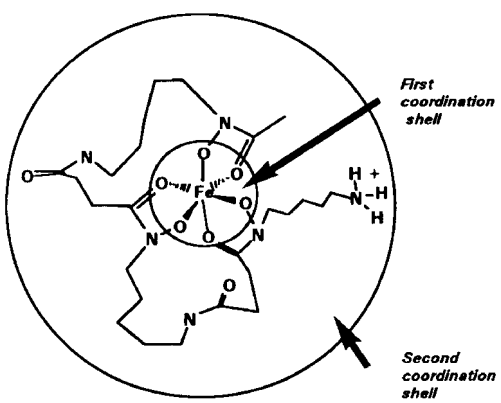
Abstract An investigation of second coordination shell complexation of ferrioxamine B by crown ethers, natural ionophores, and other macrocycles through host-guest complexation of the pendant protonated amine group is presented. The influence of guest structure, host structure, solvation shell, and counter ion on host-guest assembly stability is discussed. Application of this form of molecular recognition to bulk liquid membrane transport of a stable metal complex is also presented. The significance of ionophore recognition of a metal complex in the context of iron bioavailability is discussed.

INTRODUCTION

We are investigating the chemistry of the second coordination shell of stable transition metal complexes. Specifically, we are interested in structural features on the surface of a stable metal complex which can act as a guest in a host-guest assembly involving an ionophore. This process leads to ionophore recognition of a whole molecule, rather than just ion recognition. For our prototype system we are investigating second coordination shell molecular recognition of the iron-specific chelate complex ferrioxamine B ($FeHDFB^+$, 1). The deferriferrioxamine B ligand (H_4DFB^+), a linear trihydroxamic acid, expresses a selectivity for complexation of +3 metal ions, particularly Fe(III). Another feature of the deferriferrioxamine B structure is a pendant amine group, which is protonated at neutral pH (pK_a = 10.79 (1)) and not coordinated to the metal. This amine site has the potential of forming a host-guest complex with an appropriate crown ether or other ionophore. Our prototype system provides a combination of metal ion selectivity and molecular recognition which leads to a supramolecular assembly. This is illustrated in 2 for a ferrioxamine B complex with dicyclohexano-18-crown-6 (2).



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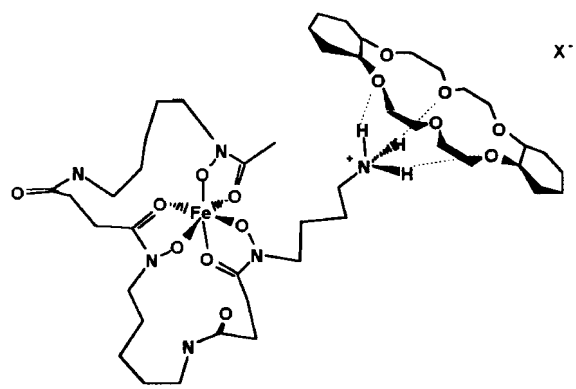

2

Ferrioxamine B is of particular interest due to its role as a siderophore. Siderophore mediated microbial acquisition of the essential element iron involves selective chelation of environmental Fe(III)

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followed by transport and deposition at the cell surface or cell interior (3,4). Metal-ligand exchange at the cell surface, or penetration of the siderophore complex into the cell interior undoubtedly involves molecular recognition by a receptor. Consequently, model chemical investigations involving second-sphere molecular recognition of siderophores has application to an understanding of iron bioavailability in microbes. Since host-guest complexation of the protonated amine side chain of ferrioxamine B increases the hydrophobicity of the complex (2), our model studies also have application to metal extractions, precious and trace metal recovery, and environmental remediation.

Our use of ferrioxamine B also provides an opportunity to study the influence of ionophore structure on host-guest complexes involving a substituted amine guest. The structure in **1** may be viewed as a substituted amine with a tris(hydroxamato)iron(III) chromophore attached. This provides a spectroscopic means to study host-guest complexation and bulk liquid membrane transport.

We are interested in developing an understanding of the basis of molecular recognition in the second coordination shell, which in the case of ferrioxamine B involves RNH_3^+ H-bonding, ion-dipole interactions and the possibility of host interaction with the ligand backbone (*e.g.* the amide linkages). Since the ferrioxamine B guest ($FeHDFB^+$) is a complex cation, we have investigated the influence of the charge density of M(III) on host-guest interaction in a series of metal substituted ferrioxamine B complexes ($MHDFB^+$). Since host-guest complex formation in our studies occurs in non aqueous medium and the assembly carries a net positive charge, we are interested in the influence of the anion in the ion pair (see **2**) on host-guest complex stability. Both M(III) and X^- are hydrophilic and are associated with a hydration shell, which will influence events in the second coordination sphere of ferrioxamine B. We will also address the influence of host structure and solvent on host-guest assembly stability. An understanding of these features will be applied to the development of a bulk liquid membrane transport system.

FACTORS INFLUENCING SECOND-SPHERE HOST-GUEST ASSEMBLY FORMATION

The host-guest association equilibrium of interest for a protonated amine guest (RNH_3^+) and crown ether host (CE) in water saturated chloroform is defined in Eq (1). Data for host-guest association

$$(RNH_3^+,X^-)_{org} + CE_{org} \overset{K_a}{<====>} (RNH_3^+,CE,X^-)_{org} \qquad (1)$$

stability constants are given in Table 1 for various R groups and the dicyclohexano-18-crown-6 host (**3a**) with picrate anion in $CHCl_3$ solution at 25 °C (2). Of prime significance is the fact that **3a** recognizes ferrioxamine B through second-sphere host-guest complexation with a log K_a value of 3.67.

TABLE 1. *Host-guest association constants (K_a) for various protonated amine guests and dicyclohexano-18-crown-6 host in wet $CHCl_3$.**

Protonated amine	log K_a
1. NH_4^+	7.69
2. $CH_3NH_3^+$	6.91
3. $(CH_3)_3CNH_3^+$	5.12
4. $CH_3(CH_2)_4NH_3^+$	6.16
5. H_4DFB^+	4.56
6. $InHDFB^+$	3.92
7. $FeHDFB^+$	3.67
8. $GaHDFB^+$	3.59
9. $AlHDFB^+$	3.48

* Data obtained by a $H_2O/CHCl_3$ liquid-liquid extraction method with picrate anion at 25 °C; ref (2).

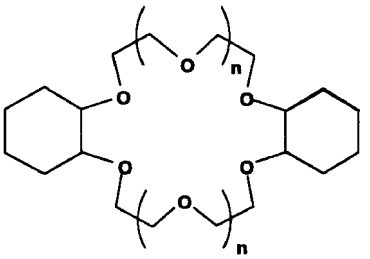
TABLE 2. *Bulk liquid membrane flux for various lipophilic ionophore carriers.**

Ionophore	Flux/mole cm^{-2} s^{-1}
6a	4.26×10^{-12}
3b	5.04×10^{-12}
10	6.35×10^{-12}
4a	2.66×10^{-11}
3a	1.90×10^{-10}
9	2.78×10^{-10}
5	2.80×10^{-10}

* Conditions: source phase - 2 mL H_2O containing 2 mM $FeHDFB^+$, 33 mM $Mg(ClO_4)_2$, pH = 4; membrane phase - 3.5 mL $CHCl_3$ containing 50 mM ionophore; receiving phase - 2 mL H_2O; ref (14).

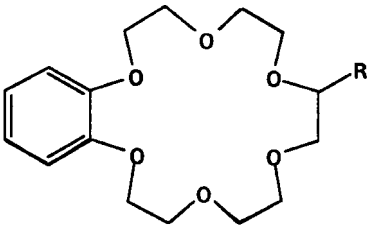
The data in Table 1 serve to illustrate the influence of H-bonding, steric hindrance and the hydration shell of the guest on host-guest complex stability. H-bonding and steric influences are illustrated by the decrease in K_a for the first three entries. Steric and hydration shell effects are illustrated by comparing the 4th and 5th entries. Addition of the hydrophilic linear tri-hydroxamic acid to the pentyl side chain has a steric effect due to the size of the chain, as well as the hydration shell associated with the hydroxamic acid groups. Incorporation of a +3 metal ion in the hydroxamate shell further decreases K_a (*c.f.* entry nos. 5 and 6-9). This decrease follows a trend with increasing charge density on M(III), which influences the solvation sheath in the second coordination shell of the metal complex. This trend illustrates the influence of the hydration shell on host-guest association in the second coordination shell.

Anion hydration also influences the stability of the supramolecular assembly illustrated in **2** (5). This is shown by the decrease in log K_a for {FeHDFB$^+$,**3a**,X^-} for different anions, which varies linearly with the hydration enthalpy of X_g^- (log K_a in parentheses): ClO_4^- (4.25); pic^- (3.67); NO_3^- (2.13); Cl^- (1.00) (5). The stability of the supramolecular assembly **2** is equally sensitive to M(III) and X^- hydration shell changes (5), suggesting that the crown ether resides in the hydration shell of both MHDFB$^+$ and X^-, although the relative positions of the anion and cation in this dynamic ion pair cannot yet be determined.



a: n = 1
b: n = 2

3



a: R = H
b: R = $CH_2O(CH_2)_4COOH$
c: R = $CH_2O(CH_2)_7COOH$

4

The position of the counter anion in the assembly **2** can be somewhat fixed by attachment to the crown ether host. In collaboration with Professor Bartsch we have investigated MHDFB$^+$ complexation by lariat ethers (**4b**, **4c**) which possess a pendant carboxylate anion (6). By investigating host-guest assembly formation at aqueous pH values above and below the pK_a of the carboxylate group we can investigate the influence of a tethered counter anion on K_a. The eight atom arm in **4b** increases log K_a by 1.08 log units and the 11 atom arm (**4c**) by 1.34 log units, relative to the corresponding benzo-crown ether (**4a**) with a free ClO_4^- anion. Clearly the tethered anion increases host-guest complex stability. The increase in stability with increasing arm length suggests the importance of facile folding of the negatively charged $-(CH_2)_nC(O)O^-$ group back into the region of the protonated amine cation guest.

Contrary to our expectation, host-guest assembly stability for ferrioxamine B and **3a** is greater in the more polar CH_2Cl_2 than in $CHCl_3$ solvent (7). The effect is more pronounced with picrate counter anion than ClO_4^- anion. This is in contrast to trends established in the literature for host-guest complexation which show that the stability of the interaction is increased by decreasing the polarity of the solvent (8).

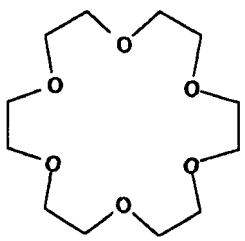
Clearly the magnitude of K_a in our systems is influenced by both cation-solvent and anion-solvent interactions. The anion-solvent influence may be summarized as follows. Changing from ClO_4^- to pic^- anion in a given water saturated organic solvent results in a decrease in K_a (5,7). This may be due to steric hindrance caused by the larger, more highly hydrated picrate anion. For a given anion, changing from $CHCl_3$ to CH_2Cl_2 solvent results in a less tightly bound ion pair, which facilitates a closer approach of the crown ether to the protonated amine side arm of ferrioxamine B. This effect offsets any increase in cation solvation by the more polar solvent, which would result in a weaker interaction with the crown ether.

Further evidence of the influence of the steric bulk of MHDFB$^+$ and its solvation shell on host-guest assembly stability can be seen in the preference of MHDFB$^+$ for *cis-syn-cis*-dicyclohexano-18-crown-6 (**3a**) over the *cis-anti-cis* isomer (9). The *syn* isomer provides two different faces above and

below the mean O atom plane. One face is sterically "open" and the other is relatively hindered. The *anti* isomer provides two faces of equivalent steric hindrance. $MHDFB^+$, whose steric bulk requires a "perched" configuration away from the mean plane of the six O atoms of **3a**, favors the *syn* isomer by 3:1, independent of M(III) and the anion. The K^+ guest, whose ionic radius is well matched to fit into the 18-crown-6 cavity and therefore is unaffected by substituents protruding above and below the O atom plane, does not express a significant preference for one isomer over the other in wet $CHCl_3$ (9).

To summarize, molecular recognition of ferrioxamine B by **3a** is sensitive to factors influencing the second coordination shell of the iron complex. This includes steric hindrance, ion pairing, and the solvation/hydration shell. We now turn to structural features associated with the ionophore host.

A number of cyclic ionophore structures are capable of second-sphere coordination to ferrioxamine B (10). Ionophore structures are shown in **3** - **8** and host-guest association constants are illustrated in Fig. 1. For the synthetic crown ethers, ferrioxamine B host-guest assembly stability decreases with increasing ring size. Host-guest complex formation is also possible with the naturally occurring cyclic antibiotics nonactin (**7**) and valinomycin (**8**).



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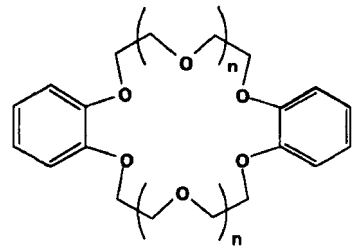
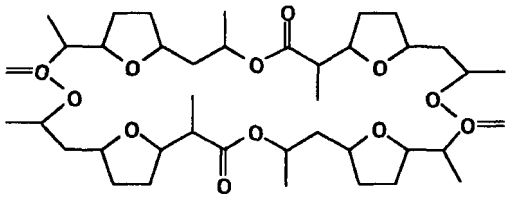


a: n = 1
b: n = 2
c: n = 3

6



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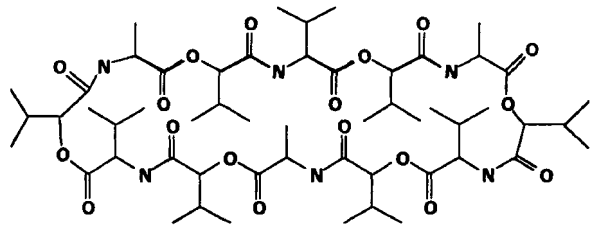


8

For ferrioxamine B, the decrease in K_a on changing the 18-crown-6 cavity substituent from two cyclohexane (**3a**) to two benzene (**6a**) rings is likely due to an increase in ring rigidity and decrease in basicity of the ether O atoms (Fig. 1). Increasing the ring size from 18 to 30 atoms (**6a**-**c**) decreases K_a. An increase in ring size and change in structure on changing from dibenzo-30-crown-10 (**6c**) to nonactin (**7**) results in an increase in stability.

Comparison of K_a values for host-guest assembly formation in the second coordination sphere of ferrioxamine B with the corresponding constants for K^+ (Fig. 1A) and NH_4^+ (Fig. 1B) guests is instructive in assessing the factors which influence ferrioxamine B host-guest complex stability. K^+ was selected for comparison due to its optimum size match for the 18-crown-6 cavity and its high affinity for valinomycin (**8**). Host-guest complex formation for K^+ involves ion-dipole interactions. NH_4^+ was selected for comparison since the ammonium ion may be considered as the parent guest for substituted amines such as ferrioxamine B. Host-guest complex formation for NH_4^+ involves H-bonding as well as ion-dipole interactions. Data presented in Fig. 1 show that both NH_4^+ and K^+ form more stable host-guest complexes with a given host than does ferrioxamine B (10). The steric bulk of the Fe(III) inner coordination shell and the solvation shell influence the host-guest interaction as noted above. The K^+ ion can nestle into the mean O atom plane of the ionophore cavity (11). The NH_4^+ ion is perched above this plane (11), and this feature is accentuated for ferrioxamine B, due to its increased steric bulk.

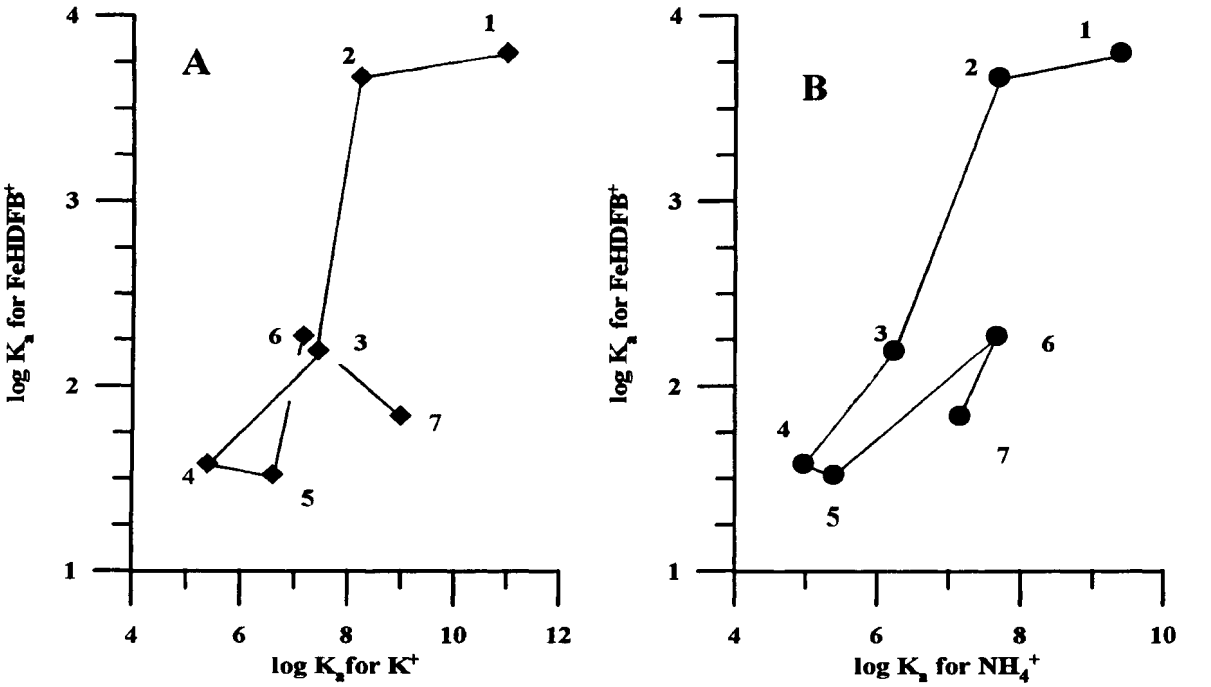
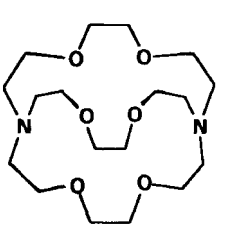


Fig. 1 *Plot of log K_a for ferrioxamine B complexation with different ionophores as a function of log K_a for the corresponding ionophore with A K^+ and B NH_4^+ guest. Data points represent the following ionophore structures: 1.* **5**; *2.* **3a**; *3.* **6a**; *4.* **6b**; *5.* **6c**; *6.* **7**; *7.* **8**. *Data obtained in wet $CHCl_3$ at 25 °C with picrate anion (2,10).*

Careful examination of Fig. 1 shows that while ferrioxamine B is insensitive to the increased 18-crown-6 ring rigidity afforded by addition of two cyclohexane ring substituents, this significantly decreases the stability of the K^+ and NH_4^+ complexes (compare data points 1 & 2). Substitution of electron withdrawing benzene rings for the cyclohexane rings decreases NH_4^+ and ferrioxamine B complexation an equivalent amount, since both guests utilize H-bonding (compare data points 2 & 3 in Fig. 1B). This substitution has a lesser effect on K^+ complexation (compare data points 2 & 3 in Fig. 1A). K^+ and NH_4^+ are more sensitive to increasing the ring size to 24 atoms than is ferrioxamine B (compare data points 3 & 4). Increasing the ring size to the highly flexible 30 atoms in **6c**, where a wrapping effect may be possible, influences ferrioxamine B stability in the opposite direction compared to NH_4^+ and K^+ (compare data points 4 & 5). The same is true for the valinomycin complex of K^+ and ferrioxamine B (data point 7 in Fig. 1A). There is no evidence for additional second-sphere interactions between the flexible valinomycin host and the ferrioxamine B backbone.

Host structures other than crown ether macrocycles are capable of second-sphere complexation of ferrioxamine B. Complexation by lariat ethers (**4b**, **4c**) is noted above. The cryptand structure [2.2.2] (**9**) forms a host-guest complex with ferrioxamine B with log K_a in the presence of ClO_4^- and pic^- anions of 4.44 and 3.00, respectively (12). This is comparable to the stability observed for **3a**, but shows a greater sensitivity to the anion present. The flexible linear antibiotic lasalocid (**10**) forms the most stable complex with ferrioxamine B; log K_a = 6.86 in the presence of ClO_4^- anion at pH 3.2 (13).



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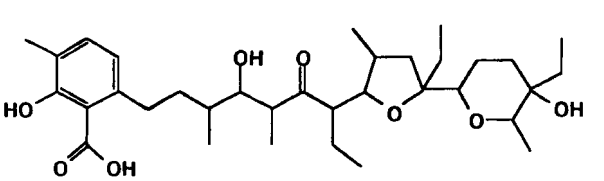

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LIQUID MEMBRANE TRANSPORT

Molecular recognition through second coordination shell complexation can also be useful for selective bulk liquid membrane transport. Use of an appropriate lipophilic ionophore enables it to perform a carrier function through a bulk liquid membrane as illustrated in Fig. 2. This system has additional application as a model for siderophore mediated biological iron transport.

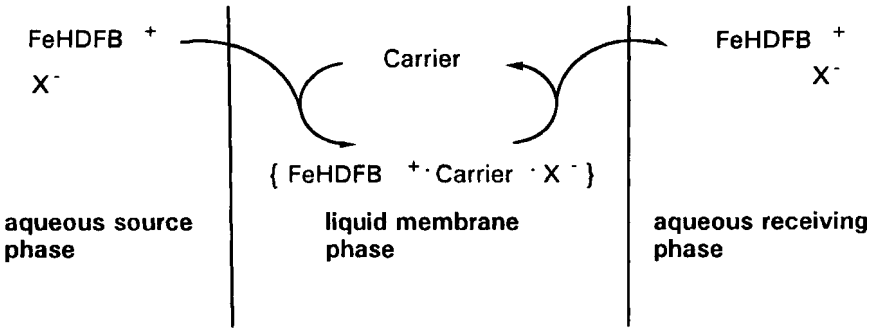

Fig 2. *Bulk liquid membrane transport. Carrier represents a lipophilic ionophore capable of recognizing ferrioxamine B through second-sphere host-guest complex formation.*

Table 2 lists bulk liquid membrane flux values for ferrioxamine B in the presence of a wide range of ionophore carriers (14). Liquid membrane fluxes are strongly ionophore structure dependent and vary over a factor of 65. These results illustrate the fact that second-sphere molecular recognition by an ionophore host can result in efficient and selective liquid membrane transport of a stable metal complex.

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