Molecular Recognition of Ferrioxamine B by Host−Guest Complex Formation with Lasalocid A in Chloroform

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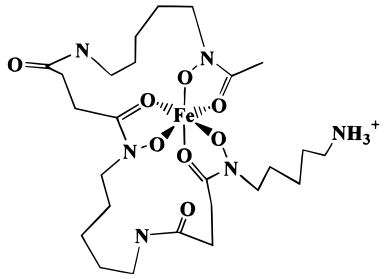
The polyether carboxylic acid antibiotic lasalocid A was employed as an ionophore in the extraction of ferrioxamine B ($FeHDFB^+$) from H_2O to $CHCl_3$ at aqueous pH 3 and pH 9. Lasalocid can selectively recognize and extract $FeHDFB^+$ in the presence of the cations Mg^{2+}, Na^+, and Li^+ through formation of a neutral second-sphere complex composed of the anionic form of lasalocid and the cationic $FeHDFB^+$. The extraction constant for the following reaction was determined to be $\log K_{ex} = -3.9 \pm 0.1$: $FeHDFB^+_{aq} + HLas_{org} \rightleftharpoons (FeHDFB,Las)_{org} + H^+_{aq}$ (K_{ex}). While lasalocid is highly effective as an anionic ionophore, it does not act as a neutral ionophore even at low pH, despite its ability to assume pseudo-crown conformations. The extraction constant for the iron-free ligand H_4DFB^+ was determined to be $\log K_{ex} = -4.4 \pm 0.2$, similar in magnitude to the constant for the complex, suggesting that the salicylate group of lasalocid does not enter the inner coordination sphere of the iron center. Lasalocid is selective for $FeHDFB^+$ by 1−3 orders of magnitude over alkali metal ions. Lasalocid is 300 times as effective as a lariat ether (benzo-18-crown-6 with a 12-atom carboxylic acid side chain) in the extraction of $FeHDFB^+$ from H_2O to $CHCl_3$ at pH 9. Since both the lariat ether and the lasalocid have easily ionizable protons to provide charge neutralization, the superiority of lasalocid is attributed to its open chain structure, which permits a stronger interaction between the backbone oxygens and the hydrogen atoms of the siderophore's pendant ammonium group.

Introduction

Siderophore-mediated microbial iron acquisition involves solubilization of environmental Fe(III) by chelation, transport to the cell surface by diffusion, selective recognition of the complex at the cell surface, and transport to the inner surface of the cell membrane as the intact complex or by Fe exchange to a membrane surface bound carrier.[1−4] Model studies, using aqueous/organic two-phase systems to simulate the interface between the cell membrane and the environment, can be used to probe the fundamental chemical aspects of the process without the complications inherent in living systems. These model systems are not meant to fully duplicate the natural ones, but provide a means for systematically examining how various chemical factors can affect the recognition and transport process. Elements that may be important in such processes include, but are not limited to, the following: first- and second-sphere coordination, hydrophobicity, solvation/desolvation, stereochemistry, steric effects, charge neutralization, and preorganization.

Synthetic crown ethers and natural antibiotics can recognize hydrophilic cations and facilitate their transfer from aqueous phases to lipophilic membranes and low-polarity organic phases.[5,6] Investigations in this laboratory have shown that the lipophilic crown ether dicyclohexano-18-crown-6 (DC18C6) can recognize the terminal protonated amine group of the iron-siderophore complex ferrioxamine B ($FeHDFB^+$, **I**) and promote



I. Ferrioxamine B ($FeHDFB^+$)

its extraction from water into chloroform through the formation of a host−guest complex.[7,8] This complex, or supramolecular assembly, is an uncharged entity consisting of the crown ether, the cationic ferrioxamine B complex, and a counteranion which

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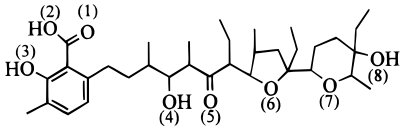
accompanies the $FeHDFB^+$ into the organic phase, maintaining electroneutrality. It was shown that the extraction is dependent on the degree of hydration of both the cation and the anion in the assembly.[7,8]

Cation binding by macrocycles can be enhanced by the addition of a sidearm chain containing additional electron donors, to form a lariat ether.[9] A lariat ether with an ionizable functionality (usually a carboxylic acid) at the end of a lipophilic sidearm chain can itself act as a counterion, obviating the need for an accompanying anion. The flexible side chain may allow a more favorable interaction between the host and the cationic guest, leading to more complete desolvation and a higher association constant.

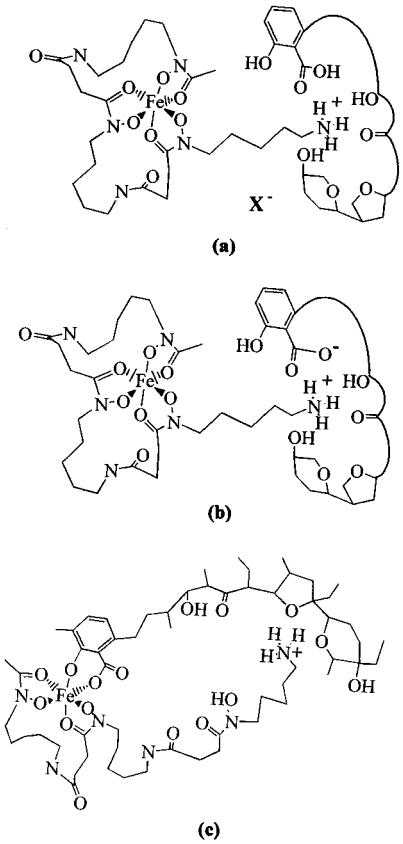
The polyether carboxylic acid antibiotics provide a rich source of new carriers for these studies. The biological activity of these natural ionophores results from their ability to break down cation gradients across cell membranes.[6c] Unlike the cyclic crown ethers, these antibiotics have open chain structures and ionizable protons which can lead to vastly different complexation behavior. Lasalocid A (**II**) was selected for study because it



II. Lasalocid A

can bind and transport protonated amines across lipid bilayer membranes and aqueous/organic phase boundaries.[10−12] In addition, lasalocid has a salicylic acid head group which is an effective iron(III) chelator.[13] This is an important feature since ternary complex formation is relevant to the release of iron from a siderophore complex by ligand exchange.[1−3] Recently, there was a report on the extraction of lanthanide acetylacetonate complexes by lasalocid via inner-sphere coordination.[14] Lasalocid has the potential to act as an ionophore for $FeHDFB^+$ in three different modes (**III**): (a) as a neutral ionophore, forming a pseudomacrocycle, its backbone oxygens interacting with the ammonium group protons in the manner of a crown ether, with coextraction of the counterion; (b) as an anionic ionophore, the salicylate group itself providing charge neutralization, similar to that of the lariat ethers; and (c) by ternary complex formation, with the salicylate group entering the inner-coordination shell of the Fe(III) center, displacing one hydroxamate group of the siderophore.

Understanding the basic chemistry of these processes is relevant to a number of applications. Siderophores can be used medically for the treatment of iron-overload diseases,[1,15] and



III. Possible binding modes in the lasalocid/$FeHDFB^+$ host-guest complex. a) Neutral ionophore. b) Anionic ionophore. c) Inner-sphere ligand.

their analogues have potential for toxic metal removal.[16] The importance of antibiotics in medicine is obvious and lasalocid has proven useful as a feed additive in the beef and poultry industries,[6c,17] while extraction by ionophores is relevant to environmental remediation, phase-transfer catalysis, and trace metal recovery. Here we report the results of our series of experiments on the extraction of $FeHDFB^+$ from water to chloroform by lasalocid A.

Experimental Section

Materials. The ferrioxamine B complex ($FeHDFB^+$) was prepared as described previously.[7] A measured amount of desferrioxamine B (H_4DFB^+, Sigma) mesylate was dissolved in about 20 mL of water, followed by the addition of iron(III) perchlorate, nitrate, or chloride stock solution as needed. The iron(III) concentration of the stock solution was determined spectrophotometrically.[18] The acid concentration of the iron(III) stock solution was determined by passing an aliquot through Dowex 50 W-X8 cation-exchange resin (H^+ form), followed by titration to a phenolphthalein end point with 0.100 M NaOH, and correcting for Fe^{3+} present. The pH of the $FeHDFB^+$ solutions was adjusted by the addition of the hydroxide appropriate to the background electrolyte cation present (LiOH(aq), NaOH(aq), or $Mg(OH)_2$(s)). In the case of $Mg(OH)_2$(s), the solid was added until the pH was slightly above the desired value. Then excess $Mg(OH)_2$(s) was filtered off and final pH adjustment was made with the appropriate acid. An ionic

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strength of 0.1 M in background electrolyte was maintained by the addition of aqueous solutions of the relevant salts. The final ferrioxamine B concentration was determined by UV/vis spectroscopy at 430 nm ($\epsilon = 2600\ M^{-1}\ cm^{-1}$).

Because the desferrioxamine B was obtained as the mesylate salt, it was necessary to determine whether the presence of the mesylate anion (Mes^-) affected the equilibria under investigation. Consequently, mesylate-free solutions of {$FeHDFB^+$, X^-} were prepared. After the iron stock solution was added to the aqueous {H_4DFB^+, Mes^-}, the resulting solution was passed through an anion exchange column (Dowex 1X8-100 resin, strongly basic) to exchange the Mes^- for the appropriate anion, ClO_4^-, NO_3^-, or Cl^-. The solution was then made to volume and adjusted for pH as described above. Results of extractions performed with the mesylate-free solutions were identical to those obtained with mesylate present. Typical concentrations were $[FeHDFB^+]_{aq} = 0.020$ M, $[X^-]_{aq} = 0.086$ M, $[Mg^{2+}]_{aq} = 0.033$ for pH 3 experiments and $[FeHDFB^+]_{aq} = 0.002$ M, $[X^-]_{aq} = 0.068$ M $[Mg^{2+}]_{aq} = 0.033$ M at pH 9; for the Li^+ and Na^+ salts at high pH, [Li^+ or Na^+] = 0.098 M, $[X^-] = 0.100$ M.

Lasalocid sodium salt (Aldrich, 97%) was converted to the acid form by a modification of the method of Juillard.[17] A chloroform solution of the sodium salt was agitated with ca. 3 M HCl, the phases were separated, and the process was repeated twice more. The chloroform solution was then washed three times with deionized water and filtered through paper (Whatman No. 1). The chloroform was removed by rotary evaporation, and the resulting greenish, viscous oil was dissolved in 2-propanol and recrystallized as fine white crystals of the lasalocid−2-propanol adduct[19] (Anal. Calcd for $C_{34}H_{54}O_8 \cdot C_3H_8O$: C, 68.28; H, 9.60; O, 22.12. Found: C, 68.18; H, 9.94; O, 21.94). The crystals were dissolved in acetone, the solution was concentrated, and the crystals were recovered. The process was repeated until fine white crystals of lasalocid acid were obtained. The product was then dried in a vacuum oven at 45 °C and 0.3 Torr for 24 h. Purity was checked by UV/vis spectroscopy at 318 nm in ethanol ($\epsilon = 4100\ M^{-1}\ cm^{-1}$).[20] Lithium lasalocid salt was prepared similarly, using 3 M LiCl. Lasalocid solutions were made by dissolving appropriate amounts in a known volume of chloroform. Because of the high volatility of chloroform, stock solutions were prepared fresh and used immediately. Lasalocid concentrations ranged from 0.0025 to 0.10 M in the pH 3 experiments and from 8×10^{-6} to 8×10^{-4} M in the pH 9 experiments.

Doubly deionized water was used throughout. Chloroform (Fisher, Spectranalyzed) saturated with water was used in distribution and extraction experiments. Ethanol, 2-propanol, and acetone were also from Fisher (Spectranalyzed). Metal salts and hydroxides used for maintaining ionic strength and adjusting pH were purchased from Aldrich.

Methods. (a) Buffer System. In a previous study it was shown that solid $Mg(OH)_2$ is an effective buffer for aqueous/organic extraction experiments at high pH due to its low solubility and minimal distribution into chloroform.[21] To compare the extraction behavior of lasalocid to that of the lariat ether, the same technique was used here for those solutions that contained Mg^{2+}; in this investigation the actual equilibrium pH ranged from 9.1 to 9.6. Solutions containing Li^+ and Na^+ were unbuffered, and the pH ranged from 8.6 to 9.1. The pH 3.2 solutions were unbuffered as well, and the pH range was 3.1−3.4. Hydrogen ion activity a_{H^+} was determined from the measured pH, and the actual measured equilibrium values were used in all calculations.

(b) Distribution Equilibria—Ferrioxamine B. Extraction equilibrium data must be corrected for the distribution of $FeHDFB^+$ and $FeDFB^0$ into chloroform in the absence of ionophore. The distribution constants ($K_d = [FeHDFB^+, X^-]_{org}/\{[FeHDFB^+]_{aq}[X^-]_{aq}\}$) of the {$FeHDFB^+$, X^-} ion pairs ($X^- = ClO_4^-$, NO_3^-, Cl^-) into chloroform have been previously determined.[8] At high pH, $FeHDFB^+$ undergoes deprotonation at the amine site ($pK_a = 10.40$)[22] to form the lipophilic uncharged species $FeDFB^0$. The equilibrium constant ($K_d = [FeDFB^0]_{org}/[FeDFB^0]_{aq}$) for distribution of $FeDFB^0$ from water to chloroform has been determined to be 1.6×10^{-2}.[21]

(c) Distribution Equilibria—Lasalocid. Lasalocid, like other polyether antibiotics, has very low water solubility[11,23−25] and so should partition almost entirely into the organic phase. Still it was deemed useful to examine the distribution behavior of lasalocid in the presence of Mg^{2+} under the conditions of this investigation. A chloroform solution of lasalocid acid (HLas) was agitated with an equal volume of 0.033 M $Mg(ClO_4)_2$ in a sealed vial. After equilibration, the phases were separated and an aliquot of the aqueous phase was reextracted with an equal volume of chloroform, the concentration of HLas being determined spectrophotometrically ($\epsilon = 3840\ M^{-1}\ cm^{-1}$ at 318 nm in $CHCl_3$). Initial HLas concentrations ranged from 0.01 to 0.24 M, and the aqueous phase pH ranged from 1.5 to 9.5. The amount of HLas distributed to the aqueous phase was either undetectable or barely detectable above background noise, so no accurate distribution constants could be calculated. In all cases, the lasalocid distributed was less than 0.05% of the total, a negligible amount. Distribution of sodium lasalocid salt into the aqueous phase has been shown to be negligible, while the lithium salt has a reported distribution quotient ($Q = [LiLas]_{aq}/[LiLas]_{org}$) of 0.153.[26] This distribution is accounted for in the equilibrium calculations for the lithium salt system.

To determine whether the presence of $FeHDFB^+$ leads to a greater partitioning of Las into the aqueous phase, a separate experiment was performed. After the extraction equilibrium was achieved, the aqueous phase was reextracted with chloroform and the Las concentration determined as before. Again, Las was barely detectable. The maximum found in the aqueous phase was at pH 9.5 and was less than 0.3% of the total Las present.

(d) Extraction Equilibria. The extraction of $FeHDFB^+$ by lasalocid was performed as described previously for crown ether and lariat ether systems.[7,8,21] At aqueous pH 3.2, the concentration of $FeHDFB^+$ in the chloroform phase was determined after it had been reextracted with 0.1 M $Ba(NO_3)_2$ into an aqueous phase and the equilibrium aqueous concentration of $FeHDFB^+$ was determined by difference. Extraction of the free ligand, H_4DFB^+, was performed in the same manner, but since the ligand has no absorption in the UV/visible range, it was necessary to perform additional steps in determining concentrations. A slight excess of Fe^{3+} stock solution was added to the reextracted aqueous solutions containing H_4DFB^+ to form $FeHDFB^+$, and the pH was adjusted to at least 7 with NaOH to ensure full coordination. Then the concentration was measured spectrophotometrically as before at 430 nm.

At pH 9, due to the large amount of $FeHDFB^+$ extracted, the concentration in the aqueous phase was measured directly, after appropriate dilution, and the organic phase concentration was determined by difference. At this high pH, $FeHDFB^+$ undergoes significant deprotonation at the amine site to form uncharged $FeDFB^0$, which is much more lipophilic than $FeHDFB^+$. Since the pK_a for the ammonium proton and the K_d for $FeDFB^0$ are known (see above), corrections for these species were made in the equilibrium calculations.

Since lasalocid is an effective ionophore for alkali and alkaline earth metal cations, extraction of these ions occurs in competition with $FeHDFB^+$ and H^+. The lasalocid extraction constants for the $H_2O/CHCl_3$ system have been determined for Li^+, Na^+,[26] and Mg^{2+},[28] and

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these were used to make appropriate corrections in the equilibrium calculations for free lasalocid in the organic phase.

UV/vis spectra were acquired using a Hewlett-Packard 8451A diode array spectrophotometer. An Orion 501 digital meter and an Orion combination electrode were used for pH measurements. FAB mass spectrometry was performed with a JEOL SX 102 spectrometer employing an acceleration potential of 10 000 V.

Results

Equilibrium Calculations. The approach used here to describe the equilibria associated with the extraction of ferrioxamine B by lasalocid is based on the general approach described in the literature for carboxylic acid antibiotics.[5] The experimental method involves the equilibration of an aqueous solution of the cation with an immiscible organic solvent containing the ionophore. Since transport and extraction usually involve the anionic carboxylate form of the ionophore,[5] the process can be described by the following equilibrium expression (eq 1), where M represents any cation, I is the ionophore, and a_H+ is the hydrogen ion activity in the aqueous phase. The

$$M^{n+}_{aq} + nHI_{org} \rightleftharpoons MI_{n,org} + nH^{+}_{aq} \quad (1)$$

extraction constant K_{ex} is expressed by eq 2. The term $y_{\pm}$ is

$$K_{ex} = \frac{[MI_n]_{org}(a_{H^+})^n}{y_{\pm}[M^{n+}]_{aq}[HI]^n_{org}} \quad (2)$$

the aqueous phase mean molar activity coefficient calculated using the extended Debye−Hückel equation.[27] For reasons of electroneutrality, release of the proton to the aqueous phase requires the transfer of some cation from the aqueous phase to the organic phase. Because solvation energies for the formation of free ions in a low-polarity solvent would be unfavorable, it is assumed that HI and MI_n are undissociated in the organic phase.[5]

The extraction equilibrium and constant for $FeHDFB^+$ are described by eqs 3 and 4, respectively. Defining a distribution ratio D_m as shown gives a linear equation (eq 5). A plot of D_m

$$FeHDFB^{+}_{aq} + HLas_{org} \rightleftharpoons FeHDFB,Las_{org} + H^{+}_{aq} \quad (3)$$

$$K_{ex} = \frac{[FeHDFB,Las]_{org}a_{H^+}}{y_{\pm}[FeHDFB^+]_{aq}[HLas]_{org}} \quad (4)$$

$$D_m = \frac{[FeHDFBLas]_{org}}{y_{\pm}[FeHDFB^+]_{aq}} = \frac{[HLas]_{org}K_{ex}}{a_{H^+}} \quad (5)$$

vs $[HLas]_{org}/a_{H^+}$ gives a straight line through the origin of slope K_{ex}, the constant for the extraction of $FeHDFB^+$ from water to chloroform by lasalocid in the acid form (Figure 1).

Extraction of $FeHDFB^+$ at pH 9. Extraction experiments were performed at aqueous pH 9 using three different background anions and Mg^{2+} as the background cation. The results were identical whether the anion was ClO_4^-, NO_3^-, or Cl^- (Figure 2). This lack of anion effect is consistent with the model proposed above, wherein the anion is not involved in the extraction equilibrium, in contrast to extraction by a neutral ionophore which exhibits a pronounced anion effect.[8]

Figure 1 is a representative plot of D_m vs $[HLas]_{org}/a_{H^+}$ showing the determination of K_{ex} in the presence of Mg^{2+}. The linearity of the plot verifies the 1:1 stoichiometry implied by eq 3. Further confirmation of the 1:1 stoichiometry is provided

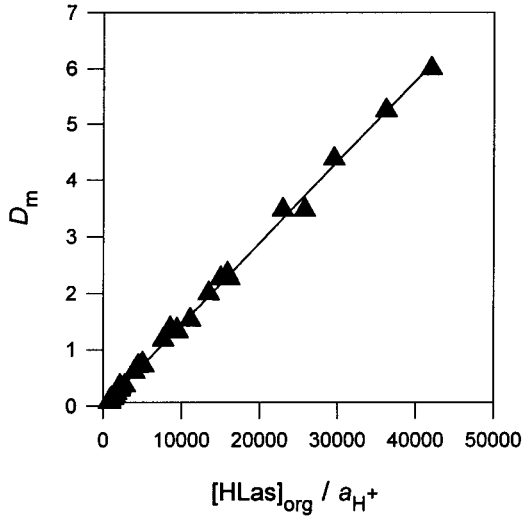

Figure 1. Plot of D_m as a function of $[HLas]_{org}/a_{H^+}$ according to eq 5 for the extraction of $FeHDFB^+$ by lasalocid into chloroform at aqueous pH 9. $I = 0.1$ M ($FeHDFB^+$, Mg^{2+}, ClO_4^-); $T = 25.0 \pm 0.1$ °C.

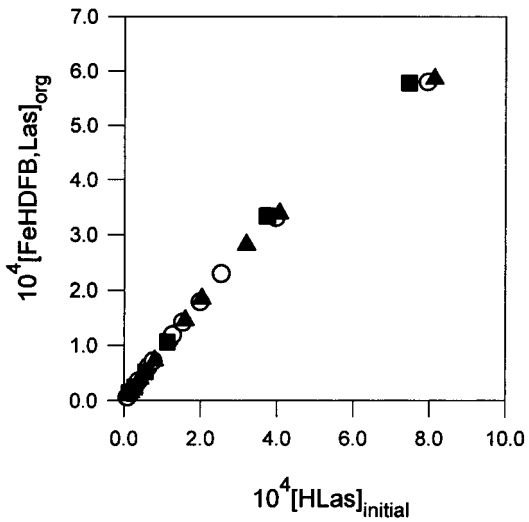

Figure 2. Plot of equilibrium ferrioxamine B concentration extracted from water to $CHCl_3$ by lasalocid ($[FeHDFB^+,Las]_{org}$) as a function of initial lasalocid acid concentration in the organic phase ($[HLas]_{initial}$) for different anions, A^-: NO_3^- (○), Cl^- (■), ClO_4^- (▲). $I = 0.1$ M ($FeHDFB^+$, Mg^{2+}, A^-); $T = 25.0 \pm 0.1$ °C. Initial $[FeHDFB^+]_{aq} = 2$ mM. pH = 9.

Table 1. Extraction Equilibrium Constants (log K_{ex}; Eq 4) for $FeHDFB^+$ and H_4DFB^+ with Lasalocid Host in $H_2O/CHCl_3$[a]

background cation	pH	log K_{ex}[b] ($FeHDFB^+$)	log K_{ex}[c] (H_4DFB^+)
Mg^{2+}	9	−3.8	
Li^+	9	−3.9	
Na^+	9	−3.8	
Mg^{2+}	3	−4.0	−4.4

[a] $T = 25 \pm 0.1$ °C; background anion ClO_4^-; calculated using eq 5. [b] ±0.1 log unit. [c] ±0.2 log unit.

by FAB mass spectrometry, obtained using a $CHCl_3$ solution extracted from a pH 9 aqueous phase and placed in a 3-nitrobenzyl alcohol matrix, which shows a strong molecular ion peak (M^+) at *m/z* 1203.7. To confirm the validity of the model, experiments were performed in the presence of Na^+, Li^+, and Mg^{2+}, cations for which the lasalocid extraction constants are known.[26,28] Table 1 displays the values determined for K_{ex} in the presence of the different cations. As required by the model

described in eqs 3 and 4, the values are the same within experimental error, regardless of the background cation.

Extraction of FeHDFB$^+$ at pH 3. In low-polarity solvents, lasalocid−cation complexes tend to assume a pseudocrown conformation, with the polar functionalities directed inward toward the cation and the nonpolar parts directed outward toward the solvent.[29] These species resemble crown ether−cation complexes, suggesting that lasalocid has the potential to act as a neutral ionophore, extracting FeHDFB$^+$ to the chloroform phase along with an accompanying anion. To test this possibility, experiments were performed at aqueous pH 3.2 (nominal), where the extraction by lasalocid anion would be low. Separate extractions were performed in the presence of three different background electrolytes, $Mg(ClO_4)_2$, $Mg(NO_3)_2$, and $MgCl_2$. In previous work with the neutral ionophore dicyclohexano-18-crown-6, the extraction constant differed by more than 3 orders of magnitude when the accompanying anion was changed from ClO_4^- to Cl^-.[8] For lasalocid, at pH 3 as at pH 9, there was no anion effect observed. This is consistent with recognition and extraction of FeHDFB$^+$ by the anionic form of lasalocid exclusively, and not by the neutral form. In addition, bulk liquid membrane transport studies at pH 3 have shown that, while lasalocid transports FeHDFB$^+$ effectively across a chloroform phase when the receiving phase contains an electrolyte solution, transport into a deionized water receiving phase is negligible, as is transport by the methyl ester of lasalocid.[30] The extraction constant K_{ex} determined at pH 3 is consistent with that determined at the higher pH (Table 1), which further supports host−guest complextion via the lasalocid anion alone.

Extraction of H$_4$DFB$^+$ at pH 3. To probe the role of the iron center and the possibility of ternary complex formation in the recognition of FeHDFB$^+$ by lasalocid, extractions were performed at pH 3 on the free siderophore ligand, H$_4$DFB$^+$. At pH 3 in the absence of Fe^{3+}, the ligand is protonated at the three hydroxamic acid sites and at the pendant amine site, providing a singly positively charged entity analogous to FeHDFB$^+$ but lacking the iron center. It was not deemed useful to extract H$_4$DFB$^+$ at high pH because deprotonation at the hydroxamic acid sites (pK_a = 8.32, 8.96, 9.55)[22] would give rise to a mixture of differently charged species. The extraction constant for H$_4$DFB$^+$ (Table 1) is of the same order of magnitude as the constant for FeHDFB$^+$.

Discussion

The lasalocid anion can very effectively recognize and extract FeHDFB$^+$ from water to chloroform by forming a stable, electrically neutral, second-sphere host−guest complex. Figure 3 shows the lasalocid extraction constants for alkali metal cations[26] and FeHDFB$^+$ as a function of the ionic radius of the cation. Ionic radii for the metal cations were taken from the literature;[31] the ionic radius for FeHDFB$^+$ in the figure (1.66 Å) is an estimate based on the ratio of partial molar volumes for ammonium chloride and methylammonium chloride at infinite dilution.[32] If one considers only that part of the ammonium group likely to interact with the ionophore, the tripodal arrangement of protons, the radius may be assumed to be close to that of NH_4^+ (1.43 Å), about the same as that of Rb^+. For the alkali metals, the extraction constants increase with ionic radius until reaching a plateau with K^+, Rb^+, and Cs^+. The FeHDFB$^+$ does not conform to this trend, exhibiting an extraction constant about an order of magnitude greater than those for the three largest metal cations shown. This higher K_{ex} value for FeHDFB$^+$ is probably a result of more favorable enthalpy changes resulting from hydrogen bonding between the lasalocid oxygens and the ammonium protons of FeHDFB$^+$.

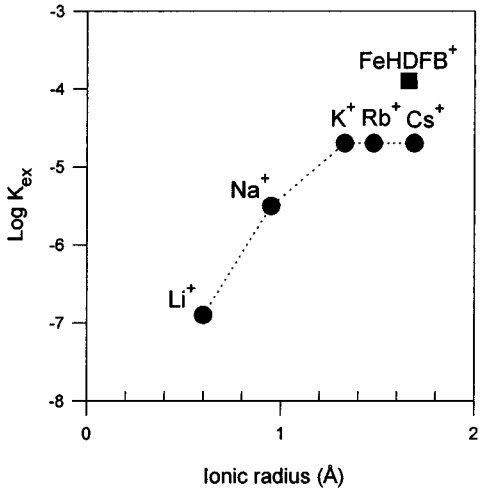

Figure 3. Plot of lasalocid $H_2O/CHCl_3$ extraction constants (log K_{ex}) for cations as a function of their radii. K_{ex} values for alkali metals are from ref 26, and cation radii are from ref 31.

The lack of an anion effect and the agreement of the K_{ex} values determined at both high and low pH provide strong evidence that lasalocid acid acts solely as an anionic ionophore for FeHDFB$^+$, exchanging H^+ for the complex across the phase boundary. This interpretation is supported by bulk liquid transport experiments which show that an exchangeable cation must be present in the aqueous receiving phase for effective transport to occur and that the methyl ester of lasalocid is unable to effect significant transport.[30] Neutral ionophore behavior by lasalocid might well be expected, as the interaction of 18-crown ethers with ammonium cations has been shown to generally involve only three oxygens[33] and lasalocid has five backbone oxygens and a high degree of conformational flexibility. Evidently the hydrogen bond stabilized, globular form[29c,34] of lasalocid acid, which must alter its conformation to enclose a cation, is simply too stable in chloroform to do so without the initial electrostatic binding to the guest provided by the carboxylate group. An 18-crown ligand, in contrast, has considerable preorganization for assembly formation with RNH_3^+ cations.[33]

No evidence has been found here for ternary complex formation. If the salicylate head group of lasalocid were able to displace a hydroxamate group from the inner-coordination sphere of Fe^{3+} the resulting ternary complex should display very different extraction behavior compared to the free ligand, which has no metal center for the lasalocid to interact with. In addition,

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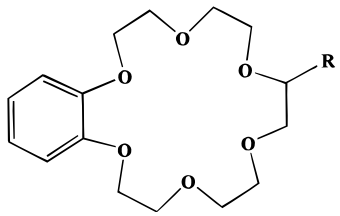
Table 2. Relative Ability of Ionophores to Extract $FeHDFB^+$ from Water to $CHCl_3$[a]

	pH 3.2[b]		pH 7.4[c]		pH 9.3[c]	
	$[ionophore]_i$[d]	rel extracting ability	$[ionophore]_i$[d]	rel extracting ability	$[ionophore]_i$[d]	rel extracting ability
lasalocid (**II**)[e]	0.12	1.7	2.4×10^{-5}	9200	2.0×10^{-5}	11 000
$L_{12}COOH$ (**IV**)[f,g]	0.18	1.1	5.6×10^{-3}	39	5.6×10^{-3}	39
B18C6 (**III**)[g,h]	0.20	1	0.22	1	0.22	1

[a] $T = 25 \pm 0.1$ °C; $I = 0.1$ M ($FeHDFB^+$, Mg^{2+}, ClO_4^-). [b] $[FeHDFB^+]_{initial} = 20$ mM. [c] $[FeHDFB^+]_{initial} = 2$ mM. [d] Initial concentration of ionophore required to extract 1% of total $FeHDFB^+$ present. [e] Calculated using K_{ex} values from this work, corrected to actual conditions with the Debye−Huckel extended law. [f] Calculated using K_{ex} values from ref 21. [g] For pH 7.4 calculation, K_{ex} values for pH 9.3 were used. [h] Calculated using K_{ex} values from ref 41.

ternary complex formation would be expected to produce a change in the spectral characteristics of $FeHDFB^+$ and no such change was observed. The fact that K_{ex} for H_4DFB^+ differs only slightly from that of $FeHDFB^+$ (Table 1) suggests that lasalocid interacts solely, or at least primarily, with the second coordination shell of the complex, the protonated amine pendant group of the $FeHDFB^+$ (and H_4DFB^+). The amide groups in the siderophore backbone may also play a role in supramolecular complex formation although there is no evidence for this. The 0.4 log unit difference in K_{ex} may be ascribed to differing degrees of hydration between the free siderophore and the siderophore−metal complex as revealed by the distribution constants of the respective cation−picrate ion pairs in going from water to chloroform ($K_d\{H_4DFB^+\} = 0.0217\ M^{-1}$; $K_d\{FeHDFB^+\} = 0.24\ M^{-1}$).[7] Although salicylate forms stable complexes with Fe(III), the necessity for a second deprotonation at the phenolic site ($pK_{a2} > 13$ for salicylic acid,[13c] probably higher for lasalocid[35]) makes it a poor competitor for the hydroxamate group, which only undergoes one deprotonation (pK_a ca. 9) in complexing Fe(III).

Lasalocid is the most effective ionophore for $FeHDFB^+$ extraction by second-sphere complexation tested to date. Table 2 is a comparison of the extracting ability of lasalocid, benzo-18-crown-6 (**IV**), and a lariat ether $L_{12}COOH$ (**V**), which is **IV**



IV. R = H; benzo-18-crown-6
V. R = $CH_2O(CH_2)_{10}COOH$; $L_{12}COOH$ lariat ether

functionalized with a carboxylic acid pendant group. The L_{12} ether was shown to be more effective at extracting $FeHDFB^+$ than the parent crown ether and analogous L_6 and L_9 ethers.[21] Extraction constants for the ionophores in Table 2 were calculated using different models: a crown ether model for the crown and lariat ethers[7,21] and a carboxylate ionophore model[5] for lasalocid. Since the resulting extraction constants have different units, a common basis for comparison may be established by expressing an extraction ability as the calculated initial concentration of ionophore required to extract 1% of the total $FeHDFB^+$ present to the $CHCl_3$ phase under the given conditions. At pH 9.3, lasalocid is 10^4 times as effective as benzo-18-crown-6 in extracting $FeHDFB^+$ into chloroform. It is evident that, at higher aqueous pH levels, an acidic ionophore can act as an anion and will extract more efficiently than a similar neutral ionophore, as the assembly formed is charge neutral and requires no accompanying anion. This is the case for the lariat ether (**V**) which is 40 times as effective as the parent compound (**IV**) in the extraction of $FeHDFB^+$ at pH 9.3. Note that, at pH 3.2, the lariat ether (**V**) and the parent ether (**IV**) are equally effective, as expected, because the lariat remains protonated and both are acting as neutral macrocyclic ionophores. The lasalocid extracts only about as well as the ethers at this low pH because the high concentration of H^+ provides strong competition for binding to the lasalocid anion.

Charge neutralization alone does not account for the high extraction efficiency of lasalocid at high pH compared to that of the lariat, even after accounting for differences in pK_a. Lasalocid has a reported aqueous pK_a of 3.7[20] while the pK_a for $L_{12}COOH$, determined in 50% methanol/water, is 5.32.[21] In water, the pK_a of the $L_{12}COOH$ is probably lower, a good estimate is that of decanoic acid, which is 4.84,[36] about an order of magnitude less acidic than lasalocid. At pH 9.3, nearly 5 orders of magnitude above the pK_a of $L_{12}COOH$, protonation equilibria are no longer significant and charge neutralization effects should be equal for both ionophores, yet lasalocid is nearly 300 times as effective in the extraction of $FeHDFB^+$ at pH 9.3 and more than 200 times as effective at pH 7.4.

It is well-known that lasalocid backbone oxygens are involved in cation complexation.[28,29b,36] Lasalocid forms pseudocrown complexes with primary ammonium cations,[10,29a,37] alkali metals,[29c,38] and the larger alkaline earth metals.[17,20,28,39] Both lasalocid and $L_{12}COOH$ provide charge neutralization by a carboxylate group, and both provide desolvation by oxygen atoms acting as electron donors. The principal difference between the two ionophores lies in their structures. In lasalocid, the donor oxygens are arranged along a flexible open chain backbone, in contrast to the covalently bound cyclic arrangement in the lariat ether. The greater conformational freedom afforded by the open structure of lasalocid can allow for simultaneous coordination by the carboxylate oxygen and at least two of the backbone oxygens, resulting in charge neutralization and optimal hydrogen-bonding interactions. This is the arrangement seen in lasalocid binding to NH_4^+ and protonated 1-amino-1-(4-bromophenyl)ethane, where the ammonium protons are coordinated by oxygens 1, 6, and 8.[10,29a] Such interactions are

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apparently less than optimal in the case of $L_{12}COOH$. The most effective arrangement would seem to be hydrogen bonding of the three ammonium protons by three oxygens in a more or less planar fashion,[33] with one of them being the carboxylate. It is likely that the constraints imposed by the cyclic conformation of the crown ether hinder the attainment of ideal geometry when the pendant carboxylate is participating in the coordination.

Our results demonstrate that lasalocid is an effective ionophore for $FeHDFB^+$, increasing the lipophilicity of the bulky, hydrophilic complex by second-sphere coordination with the pendant ammonium group. Lasalocid is selective for $FeHDFB^+$ over alkali metal ions due to its ability to form hydrogen bonds with the ammonium protons. To act as a host, it must deprotonate and act as an anion. The resulting neutralization of charge makes the $FeHDFB^+$ cation more lipophilic by reducing the degree of hydration. However, as the comparison with the lariat ether shows, charge neutralization is not the only significant factor. The process also involves desolvation by backbone oxygens in a flexible open chain arrangement that allows maximal hydrogen-bonding interactions. It is the *combination* of these elements that accounts for the efficiency of lasalocid as an extraction agent for $FeHDFB^+$.

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