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Optimization of the Lariat Ether Carboxylic Acid Host Structure for Ferrioxamine B: Demonstration of a Second Coordination Shell Chelate Effect

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Host−guest supramolecular assembly formation constants involving the second-sphere complexation of the siderophore ferrioxamine B ($FeHDFB^+$) by a lariat ether carboxylic acid host ($L_{n+2}COOH$) in wet chloroform were obtained from liquid−liquid extractions at pH values above and below the host pK_a (∼5.3). The host−guest formation constants, K_a, determined at pH = 3.2 for the assemblies $\{FeHDFB^+,L_{n+2}COOH,ClO_4^-\}$ (n = 4, 7, 10, 15) in wet chloroform are similar to those of the parent crown ether, benzo-18-crown-6. At pH = 9.3, the lariat ethers are ionized, and this results in a more stable assembly, $\{FeHDFB^+,L_{n+2}COO^-\}$, as measured by the host−guest formation constant, K_{app}. This enhanced stability is shown to be a function of the lariat ether sidearm chain length (n = 4, 7, 10, 15) and is corroborated by molecular modeling calculations. Additionally, molecular modeling and extraction data demonstrate that there is an optimum lariat ether sidearm chain length with respect to host−guest assembly stability as measured by K_{app}. We attribute the enhanced stability effect of the ionized lariat ether in the host−guest assembly $\{FeHDFB^+,L_{n+2}COO^-\}$, relative to $\{FeHDFB^+,L_{n+2}COOH,ClO_4^-\}$ or to $\{FeHDFB^+,B18C6,ClO_4^-\}$, to a second coordination shell chelate effect.

Introduction

Microbial iron acquisition presents many interesting and complex fundamental chemical problems. This process involves the molecular recognition of an iron−siderophore complex at the cell membrane. A siderophore is a chelating agent synthesized by a microorganism for the intent purpose of iron acquisition from the environment (e.g., deferriferrioxamine B which is produced by *Streptomyces pilosus*).[1−4] The biological necessity for the production of siderophores by microorganisms arises from the indispensable role of iron in many processes in the cell, combined with the near-insolubility of iron hydroxide at pH = 7 ($[Fe_{aq}^{3+}] = 10^{-18}$ M).[5] This available concentration is too low to sustain essential metabolic processes by about 10 orders of magnitude, and, therefore, strong Fe^{3+}-specific chelators (siderophores) are needed to effectively solubilize Fe^{3+}. The cellular assimilation of iron requires a protein receptor on the cell surface which recognizes the iron−siderophore complex.[6] This recognition process may be mimicked by an ionophore.[7−9] Ionophores function in part as selective recognition agents from the fact that molecular guests (electron acceptors) bind more tightly to certain host (electron donors) molecules than others. A supramolecular host−guest assembly involving a metal complex paired with a macrocycle, or an ionophore, in the second coordination sphere may be viewed as a model for some aspects of membrane-bound protein receptors. This simplification of the siderophore−receptor recognition event allows for systematic studies designed to address the factors which affect host−guest assembly stability.

The discovery of crown ethers[10] has led to the development of the lariat ether.[11,12] These molecules incorporate a macrocyclic polyether ring, a sidearm that contains one or more donor groups, and a point of attachment (the "pivot atom") joining the sidearm with the ring. The lariat ether utilized here consists of the crown ether benzo-18-crown-6 (B18C6) with a carboxylic acid sidearm attached (Figure 1). The siderophore complex consists of ferrioxamine B (Figure 1), a trihydroxamic acid hexadentate iron(III) chelate. The crystal structure of ferrioxamine B shows that the pendant protonated amine side chain is extended and is pointing away from the amide connecting ring.[13] To the extent that solid state structures can be extrapolated to solutions, this shows that the protonated amine side chain is

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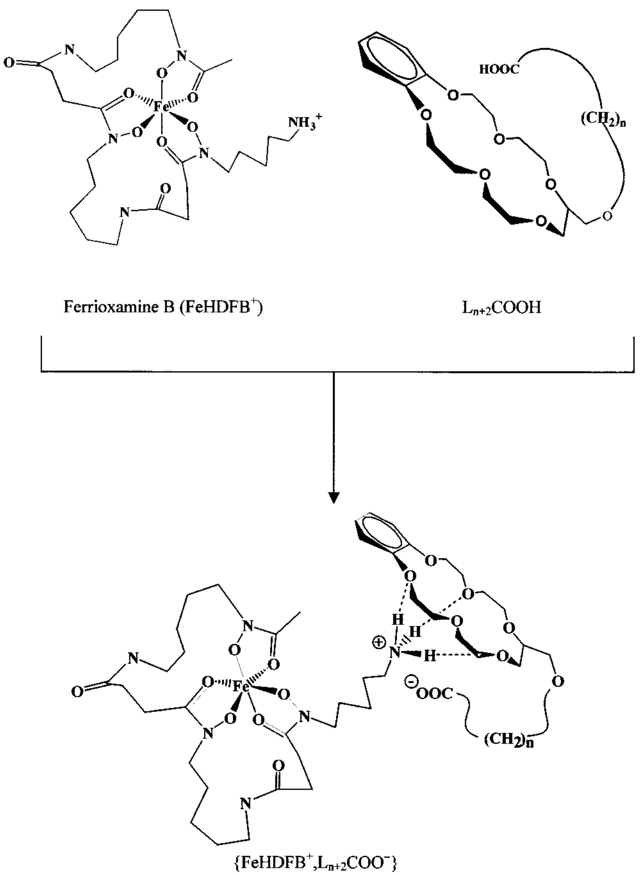

Figure 1. Ferrioxamine B and the lariat ether carboxylic acid form the host−guest assembly {FeHDFB$^+$,L$_{n+2}$COO$^-$}. Nomenclature for the lariat ether is represented as L$_{n+2}$COOH, where n denotes the number of methylene groups in the arm length and the 2 signifies the methoxy group attaching the sidearm to the crown ether; thus, $n + 2$ represents the number of atoms between the crown ether cavity and the carboxylic acid.

readily available for host−guest interactions with an ionophore. We have experimentally confirmed this assertion.[14]

At conditions where the lariat ether carboxylic acid (p$K_a \approx 5.3$) is ionized, thus obviating the need for a separate counteranion, the stability of the host−guest complex {FeHDFB$^+$,L$_{n+2}$COO$^-$} (Figure 1) is increased relative to {FeHDFB$^+$,L,ClO$_4^-$} (L = neutral ionophore).[15] We are interested in optimizing the design of an ionophore in order to maximize the stability of an ionophore−siderophore host−guest supramolecular assembly. An attached counteranion has helped us to further meet this goal,[15] and determining the optimum chain length of the sidearm on the lariat ether is the focus of this report.

This increase in host−guest assembly stability through the employment of an ionizable lariat ether ionophore can be *cautiously* attributed to a second coordination shell (with respect to the iron complex) chelate effect. First coordination shell effects with respect to lariat ethers are dealt with more explicitly in the literature and generally involve further binding of a donor atom on the lariat ether to an already complexed cation, thereby providing a secondary interaction.[16−20] To the best of our knowledge, there are few systematic investigations of the effect of the chain length on a lariat ether host−guest complex,[16] especially to the extent of exploiting an ionizable sidearm tether. This and our previous report[15] represent the first systematic investigation of second-sphere complexation as a function of lariat ether chain length. Here we discuss these results within the paradigm of a second coordination shell chelate effect.

As a guide to our synthesis and design efforts, we have used molecular mechanics calculations[21] to suggest the optimum chain length for stable ferrioxamine B/lariat ether carboxylate host−guest assemblies. Previous stability constant results have been corroborated through molecular modeling. A lariat ether with 17 atom spacers (L$_{17}$COOH; Figure 1), one that our calculations predicted would form a less stable assembly {FeHDFB$^+$,L$_{n+2}$COO$^-$} ($n = 15$) than L$_{12}$COOH, was synthesized. Experimental verification of this predicted stability trend was provided by host−guest assembly stability constant measurements.

Experimental Section

Materials. The lariat ether L$_{17}$COOH (16-[11′-(oxymethyl)-2′,3′-benzo-18-crown-6]-hexadecanoic acid) was prepared as described previously, with the exception that ethyl 16-bromodecanoic acid was used as the alkylating agent.[15] The compound was obtained as a pale yellow solid; mp 57−58 °C. UV/vis: $\epsilon = 2540$ M^{-1} cm^{-1} at 278 nm in CHCl$_3$. Anal. Calcd for $C_{33}H_{56}O_9$: C, 66.42; H, 9.46. Found: C, 66.43; H, 9.61. The composition was also confirmed by ^{1}H NMR and IR spectra.

The concentration of the lariat ether in chloroform was varied between 3.0×10^{-3} and 28×10^{-3} M. Mg(ClO$_4$)$_2$ and Mg(OH)$_2$ (Aldrich) were used for maintaining the ionic strength at 0.1 M and the desired pH of either 3.2 or 9.3. Ferrioxamine B perchlorate solutions were prepared as described previously.[14a] The concentrations of FeHDFB$^+$, ClO$_4^-$, and Mg^{2+} in the aqueous solutions were 1.9×10^{-2}, 8.0×10^{-2}, and 3.0×10^{-2} M, respectively. Doubly deionized water and reagent grade chloroform were used for each pertinent experiment.

Liquid−Liquid Extractions. Liquid−liquid extraction experiments were performed as described previously.[15] Biphasic extraction experiments were performed by vehemently shaking equal volumes (2 mL) of aqueous (at pH 3.2 or 9.3) and chloroform solutions in capped glass vials for a few minutes, followed by centrifugation and overnight equilibration at 25 ± 0.5 °C. After carefully removing the top aqueous layer, an aliquot of the organic phase was added to another glass vial filled with 1 mL of 0.1 M KClO$_4$. Due to the high affinity of the 18-crown-6 cavity of the lariat ether for K$^+$, ferrioxamine B is quantitatively displaced into the aqueous phase where its concentration was measured spectrophotometrically.[14b] UV/vis spectra were measured using a Cary 100 double-beam spectrophotometer. The equations governing the extraction, distribution, and host−guest association equilibria are presented in the Results section.

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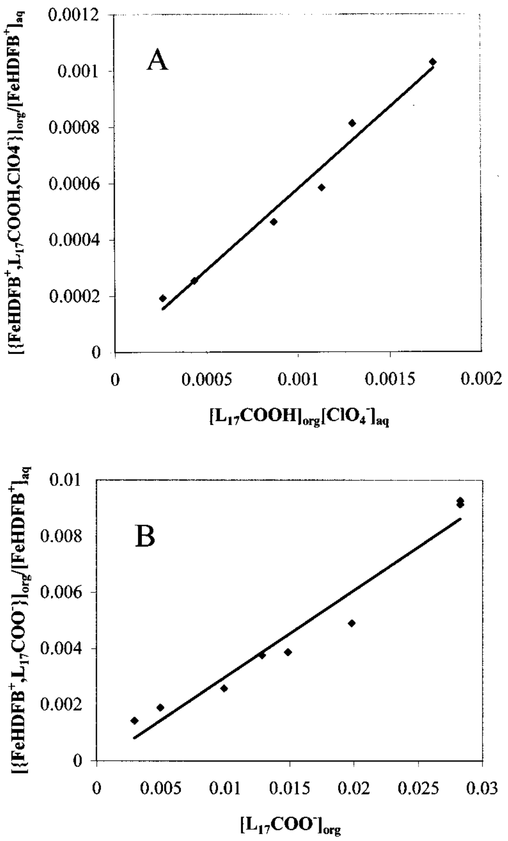

Figure 2. (A) K_{ex} determination for $\{FeHDFB^+,L_{17}COOH,ClO_4^-\}$ at pH = 3.2, according to eq 6. The slope of 0.58 M^{-2} ($R^2 = 0.98$) represents the extraction constant, K_{ex}, for equilibrium reaction 5. (B) K_{ex} determination for $\{FeHDFB^+,L_{17}COO^-\}$ at pH = 9.3, according to eq 15. The slope of 0.309 M^{-1} ($R^2 = 0.95$) represents the extraction constant, K_{ex}, for equilibrium reaction 14.

Molecular Modeling. Molecular simulations were obtained using a Silicon Graphics Indigo (SGI) workstation and SYBYL molecular modeling package[21] with the standard TRIPOS force field.[22] The calculations were carried out by invoking a simple gas-phase model, without the inclusion of solvation effects. Since calculations of metal centers are not straightforward and entail intrinsic complications, we elected to focus on the organic backbone of the metal complex, while ignoring metal torsions (van der Waals/electrostatic interactions of Fe^{3+}). Consequently, we have performed molecular modeling calculations with the protonated amine sidearm of ferrioxamine B, the pentylammonium cation ($CH_3(CH_2)_4NH_3^+$; PA^+), and the lariat ether carboxylic acid anion ($L_{n+2}COO^-$).

Results

Distribution, Extraction, and Host−Guest Equilibria. The overall extraction of ferrioxamine B ($FeHDFB^+$) by the host lariat ether from an aqueous to an organic phase serves as the experimental basis for determining the host−guest association constants, K_a or K_{app} (eqs 1 and 2, discussed below), for supramolecular assembly formation in chloroform.

$$\{FeHDFB^+,ClO_4^-\}_{org} + L_{17}COOH_{org} \overset{K_a}{\rightleftharpoons} \{FeHDFB^+,L_{17}COOH,ClO_4^-\}_{org} \tag{1}$$

$$FeHDFB^+_{org} + L_{17}COO^-_{org} \overset{K_{app}}{\rightleftharpoons} \{FeHDFB^+,L_{17}COO^-\}_{org} \tag{2}$$

The equations governing distribution, extraction, and host−guest association are represented in eqs 1−19. Extraction studies were performed at both low (3.2) and high (9.3) pH to address the effect of the lariat ether carboxylic acid ($pK_a \approx 5.3$).[15] At low pH, the lariat ether is protonated, and a separate counteranion is required in the host−guest assembly. At high pH, the lariat ether is deprotonated, and host−guest complexation consists solely of $FeHDFB^+$ and $L_{17}COO^-$. Thus, two ionophore models are required to accommodate both a neutral and an anionic ionophore.

Low pH. At pH = 3.2, the distribution of ferrioxamine B as the ion pair $\{FeHDFB^+,ClO_4^-\}$ from the aqueous to the organic phase may be expressed as in eq 3. The distribution constant is calculated according to eq 4.

$$FeHDFB^+_{aq} + ClO_4^-{}_{aq} \overset{K_d}{\rightleftharpoons} \{FeHDFB^+,ClO_4^-\}_{org} \tag{3}$$

$$K_d = [\{FeHDFB^+,ClO_4^-\}]_{org}/[FeHDFB^+]_{aq}[ClO_4^-]_{aq} \tag{4}$$

The value for K_d has been previously determined[14a] as 3.1×10^{-4} M^{-1} for an aqueous/chloroform two-phase system at 25 °C. The overall extraction equilibrium at pH = 3.2 is shown in eqs 5 and 6.

$$FeHDFB^+_{aq} + ClO_4^-{}_{aq} + L_{17}COOH_{org} \overset{K_{ex}}{\rightleftharpoons} \{FeHDFB^+,L_{17}COOH,ClO_4^-\}_{org} \tag{5}$$

$$K_{ex} = [\{FeHDFB^+,L_{17}COOH,ClO_4^-\}]_{org}/[FeHDFB^+]_{aq}[ClO_4^-]_{aq}[L_{17}COOH]_{org} \tag{6}$$

The equilibrium concentrations were calculated as follows:

$$[FeHDFB^+]_{aq} = [FeHDFB^+]^0_{aq} - [\{FeHDFB^+,L_{17}COOH,ClO_4^-\}]_{org} - [\{FeHDFB^+,ClO_4^-\}]_{org} \tag{7}$$

$$[L_{17}COOH]_{org} = [L_{17}COOH]^0_{org} - [\{FeHDFB^+,L_{17}COOH,ClO_4^-\}]_{org} \tag{8}$$

$$[\{FeHDFB^+,L_{17}COOH,ClO_4^-\}]_{org} = [FeHDFB^+]^{tot}_{org} - [\{FeHDFB^+,ClO_4^-\}]_{org} \tag{9}$$

where $[FeHDFB^+]^0_{aq}$ represents the total initial aqueous ferrioxamine B concentration, $[\{FeHDFB^+,L_{17}COOH,ClO_4^-\}]_{org}$ represents the organic phase concentration of the host−guest assembly, $[\{FeHDFB^+,ClO_4^-\}]_{org}$ represents the organic phase concentration of the ferrioxamine B perchlorate ion pair, $[L_{17}COOH]^0_{org}$ represents the total initial organic phase lariat ether concentration, and $[FeHDFB^+]^{tot}_{org}$ represents the total concentration of ferrioxamine B in the organic phase which is spectrophotometrically measured after being quantitatively re-extracted into an aqueous phase (see Liquid−Liquid Extractions). In general, the equilibrium concentration of each species consists of the difference between its initial pre-extraction concentration and its concentration in the host−guest assembly or its distribution into another phase. Plotting $[\{FeHDFB^+,L_{17}COOH,ClO_4^-\}]_{org}/[FeHDFB^+]_{aq}$ vs $[L_{17}COOH]_{org}[ClO_4^-]_{aq}$ according to eq 6 results in a straight line (Figure 2A) which confirms the stoichiometry shown in reaction 5 and permits calculation of the extraction constant from the slope ($K_{ex} = 0.58$ M^{-2}).

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After experimentally measuring the extraction constant, K_{ex}, the host−guest association constant, K_{a} (defined in eqs 1 and 10), involving supramolecular assembly formation of ferrioxamine B with the neutral lariat host in the organic phase, can be calculated from extraction and distribution data, according to eq 11. Thus, log K_{a} is 3.26.

$$K_{\mathrm{a}} = [\{\mathrm{FeHDFB}^{+},\mathrm{L}_{17}\mathrm{COOH},\mathrm{ClO}_4^{-}\}]_{\mathrm{org}}/[\{\mathrm{FeHDFB}^{+},\mathrm{ClO}_4^{-}\}]_{\mathrm{org}}[\mathrm{L}_{17}\mathrm{COOH}]_{\mathrm{org}} \qquad (10)$$

$$K_{\mathrm{a}} = K_{\mathrm{ex}}/K_{\mathrm{d}} \qquad (11)$$

High pH. At pH = 9.3, the lariat ether carboxylic acid (pK_{a} ≈ 5.3) is deprotonated ($\mathrm{L}_{17}\mathrm{COO}^{-}$), and this negatively charged host eliminates the need for a counteranion. Consequently, the equilibrium expressions must be redefined in order to accommodate an anionic ionophore model.[15] D is the distribution ratio

$$\mathrm{FeHDFB}^{+}_{\mathrm{aq}} \overset{D}{\rightleftharpoons} \mathrm{FeHDFB}^{+}_{\mathrm{org}} \qquad (12)$$

$$D = [\mathrm{FeHDFB}^{+}]_{\mathrm{org}}/[\ \mathrm{FeHDFB}^{+}]_{\mathrm{aq}} \qquad (13)$$

of ferrioxamine B between chloroform and water at 0.08 M perchlorate ion concentration and pH = 9.3. D may be defined as $K_{\mathrm{d}}[\mathrm{ClO}_4^{-}]$ and assumes a value of 2.5×10^{-5} at our experimental conditions of $[\mathrm{ClO}_4^{-}] = 0.08$ M.[15] The overall extraction equilibrium at pH = 9.3 is represented in eqs 14 and 15.

$$\mathrm{FeHDFB}^{+}_{\mathrm{aq}} + \mathrm{L}_{17}\mathrm{COO}^{-}_{\mathrm{org}} \overset{K_{\mathrm{ex}}}{\rightleftharpoons} \{\mathrm{FeHDFB}^{+},\mathrm{L}_{17}\mathrm{COO}^{-}\}_{\mathrm{org}} \qquad (14)$$

$$K_{\mathrm{ex}} = [\{\mathrm{FeHDFB}^{+},\mathrm{L}_{17}\mathrm{COO}^{-}\}]_{\mathrm{org}}/[\mathrm{FeHDFB}^{+}]_{\mathrm{aq}}[\mathrm{L}_{17}\mathrm{COO}^{-}]_{\mathrm{org}} \qquad (15)$$

The equilibrium concentrations were calculated as follows:

$$[\mathrm{FeHDFB}^{+}]_{\mathrm{aq}} = [\mathrm{FeHDFB}^{+}]^{0}_{\mathrm{aq}} - [\{\mathrm{FeHDFB}^{+},\mathrm{L}_{17}\mathrm{COO}^{-}\}]_{\mathrm{org}} - [\mathrm{FeDFB}^{0}]_{\mathrm{org}} - [\mathrm{FeHDFB}^{+}]_{\mathrm{org}} \qquad (16)$$

$$[\mathrm{L}_{17}\mathrm{COO}^{-}]_{\mathrm{org}} = [\mathrm{L}_{17}\mathrm{COOH}]^{0}_{\mathrm{org}} - [\{\mathrm{FeHDFB}^{+},\mathrm{L}_{17}\mathrm{COO}^{-}\}]_{\mathrm{org}} - [\mathrm{L}_{17}\mathrm{COO}^{-}]_{\mathrm{aq}} \qquad (17)$$

$$[\{\mathrm{FeHDFB}^{+},\mathrm{L}_{17}\mathrm{COO}^{-}\}]_{\mathrm{org}} = [\mathrm{FeHDFB}^{+}]^{\mathrm{tot}}_{\mathrm{org}} - [\mathrm{FeDFB}^{0}]_{\mathrm{org}} - [\mathrm{FeHDFB}^{+}]_{\mathrm{org}} \qquad (18)$$

where $[\mathrm{FeHDFB}^{+}]^{0}_{\mathrm{aq}}$ represents the total initial aqueous ferrioxamine B concentration, $[\{\mathrm{FeHDFB}^{+},\mathrm{L}_{17}\mathrm{COO}^{-}\}]_{\mathrm{org}}$ represents the organic phase concentration of the host−guest assembly, $[\mathrm{FeDFB}^{0}]_{\mathrm{org}}$ represents the organic phase concentration of deprotonated ferrioxamine B (calculated using the pK_{a} and distribution of deprotonated ferrioxamine B[15,23]), $[\{\mathrm{FeHDFB}^{+},\mathrm{ClO}_4^{-}\}]_{\mathrm{org}}$ represents the organic phase concentration of the ferrioxamine B perchlorate ion pair, $[\mathrm{L}_{17}\mathrm{COOH}]^{0}_{\mathrm{org}}$ represents the initial organic phase lariat ether concentration, and $[\mathrm{FeHDFB}^{+}]^{\mathrm{tot}}_{\mathrm{org}}$ represents the total concentration of ferrioxamine B in the organic phase which is spectrophotometrically measured after being quantitatively re-extracted into an aqueous phase (see Liquid−Liquid Extractions). Plotting $[\{\mathrm{FeHDFB}^{+},\mathrm{L}_{17}\mathrm{COO}^{-}\}]_{\mathrm{org}}/[\mathrm{FeHDFB}^{+}]_{\mathrm{aq}}$ vs $[\mathrm{L}_{17}\mathrm{COO}^{-}]_{\mathrm{org}}$ according to eq 15 results in a straight line (Figure 2B) which confirms the stoichiometry shown in reaction 14 and permits calculation of the extraction constant from the slope ($K_{\mathrm{ex}} = 0.31\ \mathrm{M}^{-1}$).

Table 1. Host−Guest Association Equilibrium Constants for Ferrioxamine B with Crown Ether and Lariat Ether Carboxylic Acid Hosts in Chloroform at 25 °C and $I = 0.1$ M[a]

crown or lariat ether	Log K_{a}[b] pH = 3.2	log K_{app}[c] pH = 9.3
B18C6	3.21	3.17
$\mathrm{L}_6\mathrm{COOH}$	3.38	4.29
$\mathrm{L}_9\mathrm{COOH}$	3.18	4.55
$\mathrm{L}_{12}\mathrm{COOH}$	3.26	4.85
$\mathrm{L}_{17}\mathrm{COOH}$	3.26	4.09

[a] Data for B18C6, $\mathrm{L}_6\mathrm{COOH}$, $\mathrm{L}_9\mathrm{COOH}$, and $\mathrm{L}_{12}\mathrm{COOH}$ are from ref 15. [b] Defined in eqs 1 and 11. [c] Defined in eqs 2 and 20.

After experimentally measuring the extraction constant, the apparent host−guest association constant, K_{app} (defined in eqs 2 and 19), involving supramolecular assembly formation of ferrioxamine B with the anionic lariat host in the organic phase, can be calculated from extraction and distribution data, according to eq 20. The use of an apparent association constant (K_{app} vs K_{a}) arises due to the fact that in the anionic model, we use a distribution ratio, D, as opposed to a distribution constant (cf. eqs 3 and 12) defined at a fixed $[\mathrm{ClO}_4^{-}] = 0.08$ M. The log K_{app} calculated from eq 20 is 4.09.

$$K_{\mathrm{app}} = [\{\mathrm{FeHDFB}^{+},\mathrm{L}_{17}\mathrm{COO}^{-}\}]_{\mathrm{org}}/[\mathrm{FeHDFB}^{+}]_{\mathrm{org}}[\mathrm{L}_{17}\mathrm{COO}^{-}]_{\mathrm{org}} \qquad (19)$$

$$K_{\mathrm{app}} = K_{\mathrm{ex}}/D \qquad (20)$$

Host−guest supramolecular assembly formation constants (K_{a} and K_{app}) at low (pH = 3.2) and high (pH = 9.3) pH which correspond to equilibrium reactions 1 and 2 are listed in Table 1.

Molecular Modeling. Molecular modeling was used as a guide in our synthetic efforts to design a lariat ether carboxylic acid host with an optimum chain length for ferrioxamine B host−guest assembly formation. Molecular modeling was performed using only that portion of the ferrioxamine B molecule that participates in host−guest complex formation with the lariat ether, the pendant pentylammonium (PA^{+}) side chain. This avoids unnecessary complications associated with choosing the appropriate parameters for the iron(III) inner coordination sphere. Thus, minimizations were performed on the $\{\mathrm{PA}^{+}/\mathrm{L}_{n+2}\mathrm{COO}^{-}\}$ supramolecular assemblies. Inspection of the host−guest assembly (as shown in Figure 1) reveals that the sidearm of the lariat ether carboxylic acid can adopt multiple configurations, of which the “up” and “down” configurations, shown in Figure 3, represent the two extreme conformers. That is, the lariat ether anionic tether can achieve charge stabilization by perching on top of the crown ether cavity on the opposite side relative to the alkylammonium guest (“up”) or by nestling next to the protonated pendant amine side chain of ferrioxamine B (“down”). For example, the down configuration is shown in Figure 1.

The strain energies ($-E$) were calculated for the up and down assemblies of $\{\mathrm{PA}^{+}/\mathrm{L}_{n+2}\mathrm{COO}^{-}\}$ for $n = 1$ to $n = 18$ and are plotted in Figure 3. According to this graph, the host−guest assembly strain energy varies with lariat ether chain length and is minimized when $n + 2 = 12$ for the “up” conformer and

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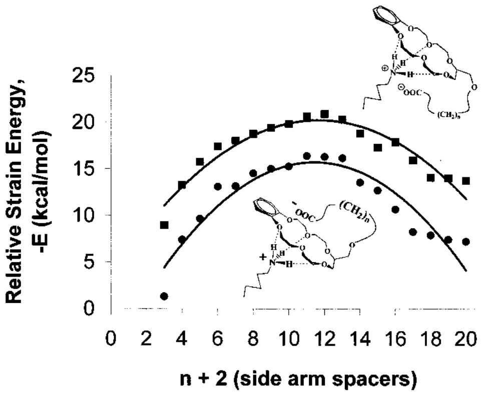

Figure 3. Plot of the relative strain energy, $-E$, for the host−guest assembly $\{PA^+,L_{n+2}COO^-\}$ obtained through molecular modeling calculations as a function of the lariat ether sidearm length, $n + 2$ (number of atoms between the crown ether host ring and the carboxylate moiety). Top and bottom plots show the “down” and “up” configurations, respectively, of the host−guest assembly. The *y*-axis was chosen such that the strain energy is an arbitrary scale.

when $n + 2 = 11$ for the “down” conformer. The minimization is greater for the “down” conformation.

The assembly stabilization energy is provided by alkylammonium H-bonding to the ether oxygen atoms and by the charge neutralization of the lariat ether carboxylate anion and the positively charged PA^+. Variations in stabilization occur through the bending of the lariat ether sidearm chain in order to more closely approach the protonated amine that sits just out of the plane of the crown ether. Once this stabilization has occurred, any extra methylene groups contribute nothing to the stability of the host−guest assembly. In fact, increasing the sidearm length starts to hinder the charge stabilization (and hence increase the strain energy, *E*) by providing fewer degrees of freedom that are germane to a longer chain. Our modeling calculations suggest maximum carboxylate lariat ether−ferrioxamine B host−guest assembly stability when $n + 2 = 12$ (±1) for $L_{n+2}COO^-$, and further suggest the pentylammonium arm is likely in the “down” configuration (Figure 1).

Discussion

Comparison of the host−guest association equilibrium constants (K_a) for the lariat ethers and the parent crown ether benzo-18-crown-6 (B18C6) at pH = 3.2 allows us to examine the influence of the presence of a pendant undissociated carboxylic acid. The low pH data in Table 1 (which are visually depicted in the lower plot of Figure 4) reveal that the nonionized lariat ether sidearm has no influence on host−guest assembly stability; that is, the stability constants are similar for B18C6 and for all of the lariat ethers. At pH = 9.3, the lariat ether carboxylic acid is deprotonated and serves as both a host and a counteranion, forming the assembly $\{FeHDFB^+,L_{n+2}COO^-\}$, as shown in Figure 1. This results in a higher host−guest association equilibrium constant (cf. log K_a at pH = 3.2 and log K_{app} at pH = 9.3 in Table 1) which varies with lariat ether chain length. Comparison of the data at high and low pH establishes that fluctuations in the assembly stability constants are due to the sidearm itself.

The K_{app} data reported here demonstrate that the lariat ether chain length passes through a maximum with respect to supramolecular assembly stability (top plot in Figure 4) as predicted by our molecular modeling results (Figure 3). This is an excellent example of a symbiotic relationship between modeling and experimental data. The molecular modeling parabola shows that as more methylene groups are added to the lariat ether sidearm past 12 atom spacers, the host−guest assembly stability begins to decrease. At 17 atom spacers, the stability (as measured in strain energy, $-E$; Figure 3) is well below that of the lariat ether with six atom spacers. This behavior is also exhibited by our experimental data, which reveals that the 17 atom spacer lariat ether lies well below its six atom spacer congener.

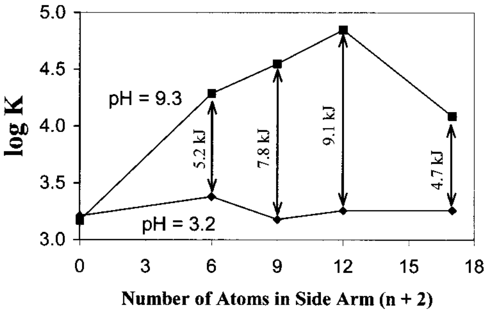

Figure 4. Plot demonstrating a second coordination shell chelate effect. The top plot is for the host−guest equilibrium (log K_{app}) to form the supramolecular assembly $\{FeHDFB^+,L_{n+2}COO^-\}_{org}$ and corresponds to eq 2. The bottom plot corresponds to log K_a for eq 1 to form the supramolecular assembly $\{FeHDFB^+,L_{n+2}COOH,ClO_4^-\}_{org}$. The *x*-axis corresponds to the number of atoms in the lariat ether ($L_{n+2}COOH$) sidearm, $n + 2$. The data point for $n + 2 = 0$ corresponds to the supramolecular assembly with the crown ether B18C6 which has no sidearm, $\{FeHDFB^+,B18C6,ClO_4^-\}_{org}$. The differences in stability constants (K_{app} and K_a) for each lariat ether were converted into ΔG values (vertically labeled in kJ/mol) that correspond to equilibrium reaction 22.

These strain energies from molecular modeling, which are tantamount to ΔG values, consist of bonding energies, valence energy interactions, torsional angle interactions, nonbonded interactions, electrostatic interactions, out-of-plane deformation energies, and hydrogen-bonding interactions. However, by altering the sidearm length, it might be argued that out of all of the strain energy terms, only the torsional angle interactions and the out-of-plane deformation energies are significantly affected. These terms are entropic in nature, and, hence, we cautiously attribute the variations in host−guest stability (K_{app}) to ΔS effects.

The longer the sidearm, the better the counteranion can approach the protonated pendant amine of ferrioxamine B which is H-bonded to the lariat ether cavity. This charge neutralization occurs through extensive bending of the sidearm, and, hence, an increase in arm length (i.e., atom spacers) leads to more facile ion pairing. Increasing the number of atom spacers proves to be advantageous up to a certain extent (here around 12). Once this optimum is reached, the sidearm can no longer better approach the amine site, and there is no increase in conformational freedom. Excess methylene groups in the arm serve the purpose now of hindering host−guest interaction, and the decrease in K_{app} reflects this. The chain length of the lariat ether carboxylate in the host−guest assembly is optimized at 12 atom spacers. It is not known whether the lariat ether sidearm curls up to hover over the crown ether cavity, or whether it curls down to the side to satisfy charge neutralization (as arbitrarily shown in Figure 1); however, molecular modeling suggests that the “down” conformer is more stable (Figure 3).

Analysis of the host−guest assembly formation constant, K_{app}, allows us to quantify the “tethering effect” of the lariat ether, which we define as the additional stability obtained by attaching the carboxylic acid to the B18C6 cavity. Since the lariat ether has an attached carboxylic acid chain, this chain is already in the second coordination shell of the ferrioxamine B complex and is less solvated than its nonattached congeners. Hence, we hypothesize that the enhanced stability of the lariat ether host−guest complex is based on a less negative ΔS value, leading to a more favorable free energy change on assembly formation. Due to this argument's comparison with the traditional "chelate effect", we term this a second coordination shell (with respect to Fe) chelate effect (SCSCE).

To more fully explore the concept of a second coordination shell chelate effect, it is helpful to look at a comparison of the energetics (ΔG) for first and second coordination shell chelate effects. The classical chelate effect (first coordination shell) is illustrated by an ethylenediamine (en) ligand exchange with ammonia at Cu^{2+} in aqueous solution (eq 21), where $\Delta G = -16.5$ kJ/mol.[24] That is, the first coordination shell chelate ring

$$\mathrm{Cu(NH_3)_2}^{2+} + \mathrm{en} \rightleftharpoons \mathrm{Cu(en)}^{2+} + 2\mathrm{NH_3} \qquad \log K = 2.9 \tag{21}$$

formation produces a more stable complex. This type of analysis can also be extended to the second coordination shell chelate effect for the lariat ether−ferrioxamine B systems studied here, where ΔG for eq 22 is calculated to be −9.1 kJ/mol for $L_{12}COO^-$. That is, second coordination shell ring formation

$$\{\mathrm{FeHDFB^+,B18C6,X^-}\}_{\mathrm{org}} + \mathrm{L}_{n+2}\mathrm{COO^-} \rightleftharpoons \{\mathrm{FeHDFB^+,L}_{n+2}\mathrm{COO^-}\}_{\mathrm{org}} + \mathrm{B18C6} + \mathrm{X^-} \qquad \log K = 1.59\ (n = 10) \tag{22}$$

produces a more stable supramolecular assembly. The results of this and of analogous calculations for the series of $L_{n+2}COO^-$ hosts are shown in Figure 4, where the vertical arrows represent a quantification of the SCSCE expressed in kJ/mol. The magnitude of the effect is dependent on sidearm chain length and, consequently, on ring size as is the classical first coordination shell chelate effect. This variation of stability with ring size lends credence to a SCSCE, as it illustrates that the increase in assembly stability comes from ring formation and not just from tethering the counterion. Also, from the perspective of the alkylammonium guest, the K_{app} values plotted in Figure 4 for $\{FeHDFB^+,L_{n+2}COO^-\}$ assembly formation represent one of the few[15,16] systematic quantitative evaluations of the influence of lariat ether chain length on host−guest stability.

We contend that the stability difference between $\{FeHDFB^+,L_{n+2}COO^-\}$ and $\{FeHDFB^+,L_{n+2}COOH,ClO_4^-\}$ is a tethering or SCSCE and is not simply due to using a carboxylate counteranion in lieu of perchlorate. The hydration energy of acetate (a good model for the lariat ether carboxylate in terms of anion hydration properties)[25] is much higher than that of perchlorate, −405 vs −209 kJ mol^{-1}, respectively.[27] In other words, without a tether effect, the lariat ether carboxylate assembly should have a lower stability owing to the greater anion hydration of the carboxylate in the wet chloroform phase. Clearly, the added stability arising from the lariat ether tether arm more than compensates for this higher anion hydration energy.

Herein we report the concept of a second coordination shell chelate effect (SCSCE), which has been quantified both by molecular modeling and by liquid−liquid extraction experiments. The effect of lariat ether chain length on the host−guest stability constant, K_{app}, is attributed to an enhanced proximity effect. This can be understood in terms of the "effective" increased flexibility of the sidearm tether that is realized via attachment of an ionizable counteranion to a crown ether and subsequent formation of a host−guest supramolecular assembly. As the ionized sidearm tether increases in length, it is easier for the counteranion to approach the cationic guest to satisfy charge neutrality. As the length increases further, there is an entropic penalty (seen as a decrease in K_{app}) associated with "containing" the sidearm to now satisfy the constraints of charge neutrality.

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(25) The validity of using acetate (CH_3COO^-) to estimate the hydration energy of the tethered side-chain carboxylate moiety ($-(CH_2)_nCOO^-$) of the lariat ether originates from our estimate that each methylene ($-CH_2-$) group may alter the free energy of hydration by 0.69 kJ mol^{-1}.[26] For the longest chain lariat ether in our study, $L_{17}COOH$, there are 16 ($-CH_2-$) groups and one O atom in the sidearm. Assuming that the O atom may cancel the effect of one ($-CH_2-$) group, we estimate that the contribution of 15 ($-CH_2-$) groups will change the free energy of hydration by ca. 10 kJ mol^{-1}. The hydration enthalpy (ΔH_{hyd}) reported for acetate is −405 kJ mol^{-1}.[27] We estimate the ΔG_{hyd} for acetate to be ca. −377 kJ mol^{-1} based on literature reports of a 7% difference between the hydration enthalpies and hydration free energies for Cl^-, ClO_4^-, and NO_3^-.[28] The addition of up to 15 sidearm ($-CH_2-$) groups should decrease ΔG_{hyd} for acetate to ca. −367 kJ mol^{-1}. Since we are considering the increased stability of the lariat ether assembly relative to the stability of the B18C6 assembly, that is, the effect of placing a tethered carboxylate group on a B18C6 cavity, we do not need to consider the hydration of the crown ether ring itself. (Even if the crown ether ring were considered, the 10 ($-CH_2-$) groups of the crown ether ring would additionally diminish the hydration free energy at most to $\Delta G_{hyd} \approx -360$ kJ mol^{-1}; this is a generous estimate since the six ring O atoms would tend to negate the effect of the $-CH_2-$ groups.) Consequently, compared to Cl^-, NO_3^-, picrate, and ClO_4^- anions ($\Delta H_{hyd} = -331, -297, -226$, and −209 kJ/mol respectively[27]), we expect the lariat ether carboxylates to be significantly hydrated in the organic phase. We estimate their partial hydration shells to be intermediate between that of Cl^- and NO_3^- in wet chloroform. Therefore, based on anion hydration shell considerations *alone*, we would expect the lariat ether assemblies $\{FeHDFB^+, L_{n+2}COO^-\}$ to be significantly less stable than $\{FeHDFB^+, B18C6, ClO_4^-\}$. This is clearly not the case which confirms our conclusion that the carboxylate tether of the lariat ether structure provides a true increase in the supramolecular assembly stability offered by the B18C6 crown ether cavity.

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