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Carrier-facilitated bulk liquid membrane transport of iron(III) hydroxamate complexes utilizing a labile recognition agent and amine recognition in the second coordination sphere †

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Carrier-facilitated bulk liquid membrane transport from an aqueous source phase through a chloroform membrane phase to an aqueous receiving phase was studied for various Fe(III) hydroxamate complexes (siderophore mimics) using second coordination sphere recognition. Iron transport systems were designed using a strategy whereby a tetradentate siderophore mimic sequesters iron(III), leaving two labile aquated coordination sites for ternary complex formation. This aquated complex reacts with a bi-functional host/guest molecule capable of acting as a host for the iron complex *via* ternary complex formation, while simultaneously serving as a guest for a membrane-bound host (carrier). The bi-functional molecules utilized contain a hydroxamate host for Fe(III) binding and a protonated primary amine that can be "recognized" by a liquid membrane-bound hydrophobic ionophore, which carries the hydrophilic Fe(III)-complex across the hydrophobic membrane to an aqueous receiving phase. Four protonated amine hydroxamic acids were investigated as bi-functional host/guest molecules: β-alanine hydroxamic acid $(H_2L^{ala})^+$, L-glutamic acid γ-monohydroxamic acid $(H_3L^{glu})^+$, glycine hydroxamic acid $(H_2L^{gly})^+$, and L-lysine hydroxamic acid $(H_3L^{lys})^{2+}$. These four bidentate ligands were each coordinated to Fe(III) along with the tetradentate *N*,*N*′-dihydroxy-*N*,*N*′-dimethyldecanediamide (H_2L^8) to form ternary complexes [$Fe(L^8)(H_xL^y)^z$; $x = 1$ or 2; y = ala, glu, gly, or lys; $z = 0$, +1, or +2] that were transported through a chloroform bulk liquid membrane by the lipophilic host carrier *cis*-dicyclohexano-18-crown-6 (DC18C6). No carrier-dependent flux was observed for $Fe(L^8)(HL^{glu})$, probably due to intramolecular H-bonding. Flux values for the transport of $Fe(L^8)(H_xL^y)^z$ ($x = 1$ or 2; y = ala, gly, or lys; $z = +1$ or +2) facilitated by the membrane carrier (DC18C6) were highest when y = gly and lowest when y = ala. Equilibrium constants pertaining to two-phase distribution or ion pairing, second coordination sphere host–guest formation, and overall extraction were determined and used to rationalize variations in flux values.

Introduction

Microbial iron acquisition is an intricate process[1–4] that starts with the synthesis and release of small selective chelators (siderophores), followed by the sequestration of iron and diffusion back to the cell surface, where the iron–siderophore complex must be recognized and, ultimately, the iron transported inside the cell for storage or use. This cellular recognition and transport event is not completely understood and poses a significant characterization challenge.[5,6] To date, two crystal structures of ferric siderophore receptor proteins (FSRP) have been reported.[7,8] The basis for molecular recognition is the ability of a membrane-bound protein to bind certain molecules more firmly than others. The resulting complex between the protein and siderophore can be viewed as a host–guest assembly, with the protein acting as the host molecule and the siderophore acting as the guest. To study the importance of various factors affecting iron recognition and transport into cells, low molecular weight ionophores, or host molecules, have been used as models for the high molecular weight membrane-bound FSRPs.[9,10]

We are developing different models to investigate molecular recognition and carrier-facilitated bulk liquid membrane (BLM) transport of iron. Two regions of the complex are used for recognition: the first and the second coordination sphere. For first coordination sphere recognition, we utilize an amphiphilic chelator, partitioned into the membrane phase, that can selectively occupy vacant or labile coordination sites on Fe(III), thus forming a hydrophobic ternary complex which can diffuse through a BLM and release the Fe(III) into the aqueous phase on the opposite side of the membrane. We have used hexadentate Fe(III) chelates with proton-facilitated partial ring opening and Fe(III) complexes with tetradentate ligands to illustrate the efficacy of this model approach.[11]

For second coordination sphere molecular recognition, two strategies for BLM transport may be envisioned. Both strategies utilize a hydrophobic host in the membrane phase which recognizes a guest moiety in the second coordination sphere of the Fe(III) complex. In the first strategy, we employ a stable hydrophilic hexacoordinate Fe(III) chelate (*e.g.* ferrioxamine B; Fig. 1) with a protonated primary amine on the surface that readily forms host–guest complexes with a variety of hydrophobic ionophores and crown ethers in the BLM phase.[12–18] The second coordination shell supramolecular assembly can diffuse through the BLM and deliver the intact hexadentate chelator to the opposite side of the membrane.[19–21] Flux values were found to be dependent on ionophore carrier structure, and a certain degree of selectivity of Fe(III) over Al(III) was demonstrated. The second strategy uses a hydrophilic bi-functional molecule with (i) a host functionality capable of coordinating to a vacant or labile coordination site on Fe(III), and thus forming a hydrophilic ternary complex, and (ii) a guest functionality capable of being recognized by a hydrophobic host. BLM transport is facilitated by ternary complex formation in the aqueous phase, followed by molecular recognition *via* second coordination sphere host–guest complexation, diffusion of the resultant hydrophobic supramolecular assembly across the BLM, and deposition of the ternary complex on the opposite side of the membrane.

This report serves to illustrate the efficacy of the second strategy described above for BLM transport *via* second coordination sphere molecular recognition. All of the ternary complexes build upon the molecular architecture of the

† Electronic supplementary information (ESI) available: Fig. S1–S6, discussed in the text. See http://www.rsc.org/suppdata/dt/b3/b306810b/

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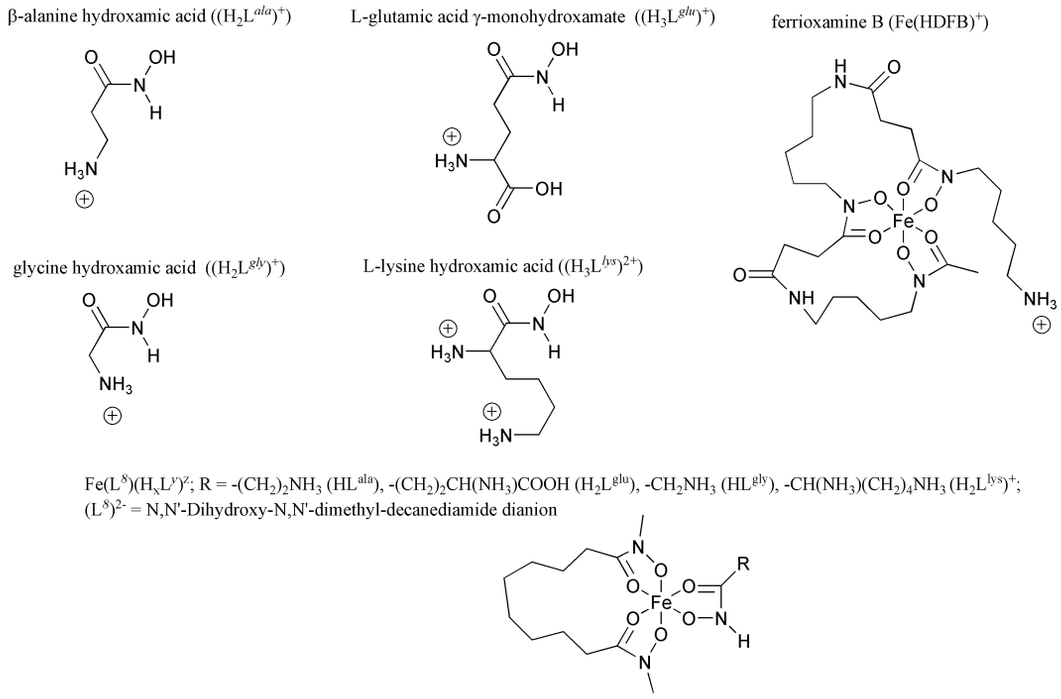

Fig. 1 Structures of the amine hydroxamic acids used herein and their ternary iron complexes.

complex between the tetradentate ligand *N*,*N*′-dihydroxy-*N*,*N*′-dimethyldecanediamide (H_2L^8) and Fe(III) [($Fe(L^8)(OH_2)_2^+$; Fig. 1]. *N*,*N*′-Dihydroxy-*N*,*N*′-dimethyldecanediamide (H_2L^8) fills four of the six coordination sites on Fe(III) and leaves two labile water ligands. These can be replaced with a series of hydrophilic bi-functional molecules, P~Q, which serve to facilitate the selective transport of a hydrophilic iron complex through a BLM. The bi-functional molecule P~Q is designed such that P is a metal-binding hydroxamic acid group and Q is a protonated amine capable of being recognized by a membrane-bound ionophore, in this case, the crown ether *cis*-dicyclohexano-18-crown-6 (DC18C6). Four molecules with the prescribed P~Q ligand architecture [protonated amine forms of β-alanine hydroxamic acid[22] $(H_2L^{ala})^+$, *L*-glutamic acid γ-monohydroxamic acid[23] $(H_3L^{glu})^+$, glycine hydroxamic acid[24–29] $(H_2L^{gly})^+$, and L-lysine hydroxamic acid[30,31] $(H_3L^{lys})^{2+}$; Fig. 1] have been used in this work, varying in amine location, overall charge, and functional groups, to complete the coordination sphere of $Fe(L^8)(OH_2)_2^+$ *via* ternary complex formation. Fig. 2 shows a generic scheme describing our approach.

Experimental

Materials

All aqueous solutions were prepared with deionized water and the pH adjusted with $Mg(OH)_2$ (Aldrich) and $HClO_4$ (Fisher, 70%). The pH was measured with a Corning 250 pH/ion meter equipped with an Orion ROSS pH electrode filled with a 3.0 M NaCl solution and standardized by two buffers. $Mg(ClO_4)_2$ (Perkin-Elmer) was used to control ionic strength. Chloroform (Mallinckrodt ChromAR®, HPLC grade) was used for the liquid membrane and *cis*-dicyclohexano-18-crown-6 (Aldrich, 98%) was used as the carrier.

N,*N*′-Dihydroxy-*N*,*N*′-dimethyldecanediamide was prepared and characterized as previously described.[32] A 0.022 M ferrioxamine B ($FeHDFB^+ClO_4^-$) stock solution was prepared as previously described.[20] β-Alanine hydroxamate hydrochloride [$(H_2L^{ala})Cl$], L-glutamic acid γ-monohydroxamate [H_2L^{glu}], glycine hydroxamate [HL^{gly}], and L-lysine hydroxamate hydrochloride [$(H_2L^{lys})Cl$] were all sourced from Sigma and used as obtained.

Methods

Aqueous solutions of $Fe(L^8)(H_xL^y)^z$ ($x = 1$ or 2; y = ala, glu, gly, or lys; $z = 0$, +1 or +2) were prepared by first dissolving equimolar amounts of H_2L^8 in an aqueous solution containing 0.112 M $Fe(ClO_4)_3$ at pH 1.0 and diluting to volume with 0.1 M $Mg(ClO_4)_2$ to induce complete solubility, followed by the addition of 10 equivalents of H_xL^y and the pH adjusted with $Mg(OH)_2$. Solutions were filtered and characterized spectrophotometrically (λ_{max} = 427 nm and ε_{max} = 2750(50) and 2800(50) cm^{-1} M^{-1} for y = ala and gly, and y = lys and glu, respectively).

Two-phase extractions were performed by vigorous manual shaking of screw-cap glass vials containing 2 mL of each of the following solutions: an aqueous solution containing 1.5 mM $Fe(L^8)(H_xL^y)^z$ and 0.1 M $Mg(ClO_4)_2$ at pH 4.6 and a chloroform solution containing 0.0–0.200 M DC18C6. After shaking for 5 min, the solutions were centrifuged and allowed to equilibrate overnight.[12] The concentration of $Fe(L^8)(H_xL^y)^z$ in each phase was calculated from the UV-vis spectra measured by a Varian (Cary 100 BIO) spectrophotometer and the total $\varepsilon_{427\ nm}$ for the combined absorbance of both solutions was checked to ensure that the complex did not dissociate or precipitate during the extraction procedure. All data points reported represent the average of three trials.

BLM transport experiments were carried out as previously described.[11,20,21] Data trials consisted of continuous absorbance readings of any of the three phases (aqueous source, membrane, and aqueous receiving) obtained using a Beckman Acta III double-beam UV-vis spectrophotometer. The source and receiving phases each consisted of a 2 mL aqueous solution, and the membrane phase consisted of a 4 mL chloroform solution. Flux values (mol cm^{-2} s^{-1}) were calculated from the absorbance *versus* time traces as previously described.[11,20,21] Flux data points represent the average of 1–3 individual replications. Uncertainties in individual flux values are in the 5–10% range.

ESI-MS spectra were obtained using an Agilent 1100 Series LC/MSD Ion Trap mass spectrometer. All experiments were performed in positive ion mode; the dry gas temperature and flow rate were 95 °C and 2.0 L min^{-1}, respectively, and the nebulizer was at 15 psi. The mobile phase was pure water at pH 4.6 and a syringe pump introduced the sample at a flow rate

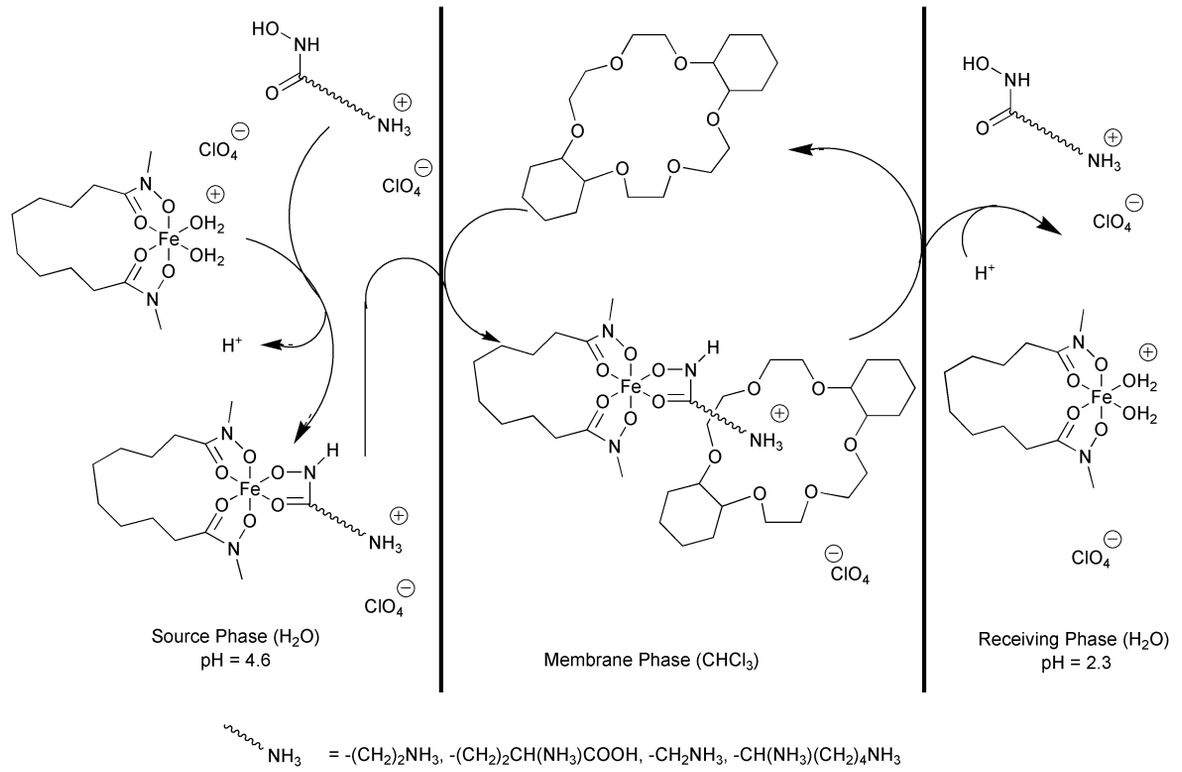

Fig. 2 General schematic representation of carrier-facilitated BLM transport of $Fe(L^8)(H_xL^y)^z$ ($x = 1$ or 2; y = ala, glu, gly, and lys; $z = 0$, +1, or +2) utilizing labile bi-functional ligands and second coordination sphere recognition by the membrane-bound ionophore DC18C6.

of *ca.* 30 μL min^{-1}. Metal to ligand ratios were the same as in the extraction experiments (1 : 1 : 10).

Results

General considerations

The basis for second coordination sphere recognition in our experiments is the presence of a protonated primary amine on the surface of all of the ternary Fe(III) complexes (Fig. 1 and 2). The protonated amine can act as an electron-pair acceptor from the crown ether and form a lipophilic host–guest assembly that drives the equilibrium towards Fe(III) extraction into the lipophilic membrane phase. The kinetics of host–guest complexation and decomplexation is rapid and physical diffusion through the unstirred membrane layers should be rate limiting.[33]

Ternary complex formation [$Fe(L^8)(H_xL^y)^z$; Fig. 1], as shown in eqn. 1, occurs under our conditions. Evidence for ternary complex formation comes from the appearance of a new UV-vis absorbance band at $\lambda_{max} \approx 425$ nm, diagnostic of tris-hydroxamate coordination to Fe(III).[1]

$$Fe(L^8)(OH_2)_2^{+} + H_{x+1}L^z \longrightarrow Fe(L^8)(H_xL^y)^z + H^+ \quad (1)$$

Confirmation of the tris-hydroxamate species as a ternary complex of composition $Fe(L^8)(H_xL^y)^z$ comes from the ESI-MS spectra, which exhibit the appropriate parent ion peak under the conditions of our BLM experiments (Fig. S1–S3, ESI†).

The overall flux of the ternary complex $Fe(L^8)(H_xL^y)^z$ in a three-phase bulk liquid membrane (BLM) transport experiment, as illustrated in Fig. 2, is controlled by two processes; the equilibria and kinetics involved in the two-phase extraction of the hydrophilic guest ternary complex from the aqueous source phase into the hydrophobic membrane phase (eqn. 2–5) and the re-extraction of the hydrophilic ternary complex molecule into the aqueous receiving phase (eqn. 6), where $x = 1$ or 2, y = ala, glu, gly, or lys, and $z = 1$ or 2 (see Fig. 1 for structures).[11,20,21]

$$Fe(L^8)(H_xL^y)^z{}_{(aq)} + zClO_4^-{}_{(aq)} \overset{K_d}{\rightleftharpoons} \{Fe(L^8)(H_xL^y)^z, zClO_4^-\}_{(org)} \quad (2)$$

$$\{Fe(L^8)(H_xL^y)^z, zClO_4^-\}_{(org)} + DC18C6_{(org)} \overset{K_a}{\rightleftharpoons} \{Fe(L^8)(H_xL^y)^z, zClO_4^-, DC18C6\}_{(org)} \quad (3)$$

$$Fe(L^8)(H_xL^y)^z{}_{(aq)} + zClO_4^-{}_{(aq)} + DC18C6_{(org)} \overset{K_{ex}}{\rightleftharpoons} \{Fe(L^8)(H_xL^y)^z, zClO_4^-, DC18C6\}_{(org)} \quad (4)$$

$$K_aK_d = K_{ex} \quad (5)$$

$$\{Fe(L^8)(H_xL^y)^z, zClO_4^-, DC18C6\}_{(org)} + H_3O^+{}_{(aq)} + H_2O_{(aq)} \rightleftharpoons Fe(L^8)(OH_2)_2^+{}_{(aq)} + zClO_4^-{}_{(aq)} + DC18C6_{(org)} + (H_{x+1}L^y)^z{}_{(aq)} \quad (6)$$

The overall extraction into the membrane phase (eqn. 4) is driven by two processes; an ion pairing and distribution process that is a direct measure of hydrophilicity/hydrophobicity (eqn. 2), and an association process that is dependent upon the thermodynamic stability of the host–guest complex (eqn. 3). In these reactions, the lipophilic crown ether (DC18C6) is the host molecule, or electron-pair donor, and the hydrophilic iron complexes are the guest molecules, or electron-pair acceptors. The host–guest assembly is stabilized by exothermic intermolecular hydrogen bonding.[17]

Equilibrium constant determination (K_d, K_a, and K_{ex})

Distribution equilibrium constants (eqn. 2) were determined for all four ternary complexes (Fig. 1, Table 1). The fraction of the ternary iron complex that partitions into the organic phase under our experimental conditions was calculated (Table 1, col. 5) in order to compare the lipophilicity of the four ternary complexes. The lipophilicity of all of the ternary complexes is comparable (Table 1, col. 5); $Fe(L^8)(HL^{ala})^+$ is the least lipophilic and $Fe(L^8)(HL^{gly})^+$ is the most lipophilic.

Table 1 Equilibrium constants for ternary iron(III) complexes and ferrioxamine B for aqueous/chloroform phase distribution, extraction and host–guest complexation

	K_d [a]	K_a [b]	K_{ex} [a,c]	%Org. [d]	$[DC18C6]_{1\%}$ [e]/mM	REA [f]
$Fe(L^8)(HL^{ala})^+$	1.34(12) M^{-1}	5.2(8) M^{-1}	7.0(8) M^{-2}	35	16	1.2
$Fe(L^8)(HL^{glu})$	0.724(10)			42		
$Fe(L^8)(HL^{gly})^+$	4.1(2) M^{-1}	5.6(4) M^{-1}	23.2(9) M^{-2}	45	6.2	3.1
$Fe(L^8)(H_2L^{lys})^{2+}$	15.7(9) M^{-2}	15.7(10) M^{-1}	246(6) M^{-3}	39	4.7	4.1
$Fe(HDFB)^+$	3.1×10^{-4} M^{-1} [g]	1.77×10^4 M^{-1} [g]	5.50 M^{-2} [g]	6.2×10^{-3}	19	1

[a] K_d corresponds to eqn. 2 and K_{ex} corresponds to eqn. 4. Conditions (aqueous phase): 0.1 M $Mg(ClO_4)_2$, pH 4.6, [Fe(III) complex] = 1.5 mM. Each value is an average of three independent determinations and the values in parentheses represent 90% confidence intervals. [b] K_a corresponds to eqn. 3. Values calculated indirectly from K_d and K_{ex} using eqn. 5. Each value is an average of three independent determinations and the values in parentheses represent 90% confidence intervals. [c] See Fig. S1–S3, ESI†. [d] Calculated percentage (from K_d) of Fe(III) complex found in pure $CHCl_3$ when allowed to equilibrate with an aqueous solution of equal volume containing the iron complex and 0.1 M $Mg(ClO_4)_2$. [e] Concentration of DC18C6 needed to bind 1% of the total iron in host–guest assembly at equilibrium when equal volumes of a solution containing 20 mM of Fe(III) complex and 0.1 M $Mg(ClO_4)_2$, and a chloroform solution containing DC18C6 are mixed. [f] Relative extracting ability—calculated from $[DC18C6]_{1\%}$ (see ref. 14). [g] Ferrioxamine B (data from ref. 12).

The overall extraction constants (K_{ex}; eqn. 4) were calculated from the slopes of plots of [host–guest assembly]/([$Fe(L^8)(H_xL^y)^z$][ClO_4^-]^z) as a function of DC18C6 concentration (Fig. S4–S6, ESI †; eqn. 4) and the host–guest association constants (K_a; eqn. 3) were calculated indirectly from the K_d and K_{ex} values (eqn. 5, Table 1). The host–guest association constants (K_a) for $Fe(L^8)(HL^{ala})^+$ and $Fe(L^8)(HL^{gly})^+$ are within experimental error of each other and indicate that unfavorable steric interactions between the crown ether host and the protonated α-amine guest caused by close proximity to the Fe(III) center are minimal. The largest host–guest association constant was found for the $Fe(L^8)(H_2L^{lys})^{2+}$ complex and could be the result of either multiple host–guest formation sites or an entropic/steric advantage due to the long flexible carbon chain on $(H_2L^{lys})^+$. The 1 : 1 host–guest stoichiometry shown in eqn. 3 is confirmed by the linearity of the plots in Fig. S4–S6 (ESI †). The $Fe(L^8)(HL^{glu})$ complex was unable to form a host–guest complex with DC18C6. This is attributed to hydrogen bond formation between the deprotonated carboxylate group ($pK_a = 2.21$) and the adjacent protonated amine ($pK_a = 9.50$) interfering with the ability of DC18C6 to hydrogen bond to the protonated amine in $Fe(L^8)(HL^{glu})$.[23]

The K_{ex} values are not directly comparable to each other as they have different units. In order to form a basis for comparison we have constructed a relative extracting ability scale (REA; Table 1, col. 7). The REA is calculated from the minimum host (DC18C6) concentration needed to bind 1% of the total iron in a host–guest assembly when equal volumes of a solution containing 20 mM of Fe(III) complex and 0.1 M ClO_4^-, and a chloroform solution containing DC18C6, are mixed.[16] The $Fe(L^8)(H_2L^{lys})^{2+}$ complex has the greatest susceptibility to extraction by DC18C6, even though it is less lipophilic than the $Fe(L^8)(H_2L^{gly})^+$ complex, as a result of its large K_a value relative to the other ternary complexes.

Bulk liquid membrane transport of the ternary complexes

Bulk liquid membrane transport facilitated by second coordination sphere recognition was successful for a series of ternary Fe(III) hydroxamate complexes shown in Fig. 1. For the three ternary complexes [$Fe(L^8)(HL^{ala})^+$, $Fe(L^8)(HL^{gly})^+$, and $Fe(L^8)(H_2L^{lys})^{2+}$], the flux increases with increasing carrier (DC18C6) concentration (Fig. 3). The BLM transport of all of the ternary complexes have a linear relationship between carrier concentration and flux at low [DC18C6] and saturation profiles are observed for $Fe(L^8)(HL^{gly})^+$ and $Fe(L^8)(H_2L^{lys})^{2+}$, the two complexes with the largest flux values, at high carrier concentration (Fig. 3, inset). No dependence on carrier concentration was observed for $Fe(L^8)(HL^{glu})^+$, which is consistent with our observation that host–guest complexation in the second sphere of this ternary complex does not occur.

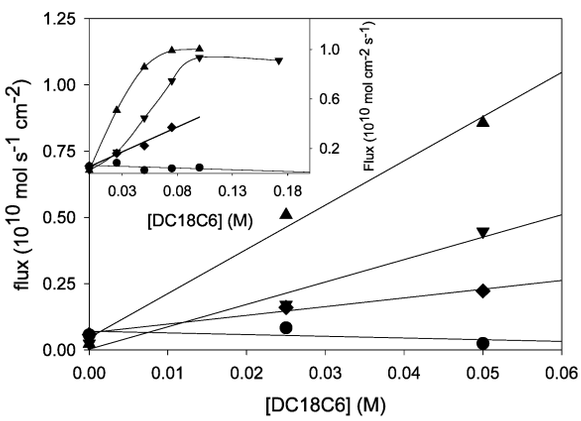

Fig. 3 Flux of $Fe(L^8)(H_xL^y)^z$ ($x = 1$ or 2; y = ala, glu, gly, or lys; $z = 0$, +1, or +2) as a function of carrier (DC18C6) concentration: (▲) $x = 1$, y = gly, and $z = 1$; (▼) $x = 2$, y = lys, and $z = 2$; (◆) $x = 1$, y = ala, and $z = 1$; (●) $x = 1$, y = glu, and $z = 0$. Source phase: 0.1 M $Mg(ClO_4)_2$, 1.5 mM $Fe(L^8)(H_xL^y)^z$, pH 4.6. Membrane phase: 0.0–0.17 M DC18C6. Receiving phase: 0.1 M $Mg(ClO_4)_2$, pH 2.3. Solid lines in the main figure represent linear least squares fits to the data. Solid lines in the inset illustrate trends in the data.

Discussion

The four ternary complexes studied in this work all contain primary amines and are tris-hydroxamate Fe(III) complexes. Three of the ternary complexes reported here [($Fe(L^8)(HL^{ala})^+$, $Fe(L^8)(HL^{gly})^+$, and $Fe(L^8)(H_2L^{lys})^{2+}$] readily form host–guest complexes in the second coordination sphere with DC18C6. The K_a values (eqn. 3) are comparable (Table 1) with $Fe(L^8)(H_2L^{lys})^{2+}$ forming the most stable ternary complex host–guest assembly by a factor of *ca.* 3. Ferrioxamine B [$Fe(HDFB)^+$] is also a tris-hydroxamate complex with a protonated amine on the surface (Fig. 1), but differs from the ternary complexes reported here in that it is a hexadentate chelate. The K_a value for ferrioxamine B is significantly larger than for the ternary complexes (Table 1). The reasons for this difference are not clear, although steric factors may be important and the high hydrophilicity of the ferrioxamine B complex may act as an additional incentive for host–guest formation with the lipophilic crown ether in organic solvent.

Three of the ternary complexes formed in this study [($Fe(L^8)(HL^{ala})^+$, $Fe(L^8)(HL^{gly})^+$, and $Fe(L^8)(H_2L^{lys})^{2+}$] exhibit significant BLM transport fluxes facilitated by DC18C6 (Fig. 3). Two observations confirm second coordination sphere recognition as the mechanism for DC18C6 carrier-facilitated BLM transport. Both the hydrophobic host DC18C6 and the bi-functional aminohydroxamic acid molecules ($H_{x+1}L^y$) must be present for the BLM transport of $Fe(L^8)(OH_2)_2^+$ to occur. [Data in Fig. 3 demonstrate that when no DC18C6 is present in

the membrane phase, there is minimal flux for $Fe(L^8)(HL^y)^z$ and the transport of $Fe(L^8)(OH_2)_2^+$ in the presence of DC18C6 is significantly less than that observed for $Fe(L^8)(HL^y)^z$.] Additionally, $Fe(L^8)(OH_2)_2^+$ was only minimally detected in the ESI-MS spectra obtained under conditions of ternary complex, $Fe(L^8)(HL^y)^z$, formation used in our BLM flux experiments, which rules out the di-aquo complex as a possible iron species being transported. All of the data indicate that the mechanism for iron transport is second sphere host–guest formation between a protonated amine on a tris-hydroxamate ternary iron complex and DC18C6.

A linear relationship between flux and K_{ex} is based on the assumption that when the distribution and host–guest association equilibria (eqn. 2 and 3) are rapidly established, the diffusion of the host–guest complex across the unstirred portion of the BLM will control the overall flux. Under these conditions, eqn. 7 relates the flux (mol cm^{-2} s^{-1}) to the extraction constant (K_{ex}), the diffusion coefficient of the complex assembly in the membrane solvent (D_{mem}), the diffusion layer or membrane thickness (l), the carrier concentration in the membrane phase ($[DC18C6]_{org}$), and the iron complex ($[Fe(L^8)(H_xL^y)^z]_{aq\ source}$) and anion ($[ClO_4^-]_{aq\ source}$) concentrations in the aqueous source phase.[11,12]

$$\text{Flux} = K_{ex}(D_{mem}/l)[DC18C6]_{org}[Fe(L^8)(H_xL^y)^z]_{aq\ source}[ClO_4^-]^z_{aq\ source} \quad (7)$$

The correlation between flux and K_{ex} predicted by eqn. 7 is observed for the ternary complexes reported here and ferrioxamine B, as illustrated in Fig. 4.[34] Fig. 4 and eqn. 7 can be used to estimate a value for D_{mem}/l (5.2×10^{-4} cm s^{-1}) that is within experimental error of the value previously calculated (5.0×10^{-4} cm s^{-1}) using different carriers and concentrations.[20] This agreement demonstrates the validity of eqn. 7 and the diffusion-controlled BLM flux model on which it is based. It is reasonable that the various host–guest supramolecular assemblies should have similar diffusion coefficients due to similarities between their structures.

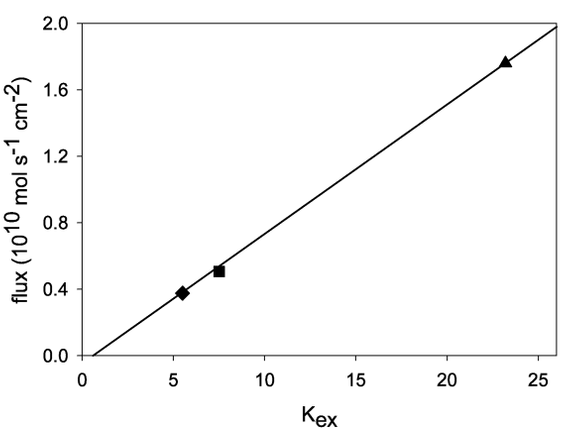

Fig. 4 Flux of $Fe(L^8)(HL^y)^+$ (y = ala, gly) and ferrioxamine B as a function of K_{ex}: (◆) $Fe(HDFB)^+$; (▲) $Fe(L^8)(HL^{gly})^+$; (■) $Fe(L^8)(HL^{ala})^+$. Source phase: 0.1 M $Mg(ClO_4)_2$, 1.5 mM Fe(III) complex, pH 4.6. Membrane phase: 50 mM DC18C6. Receiving phase: 0.1 M $Mg(ClO_4)_2$, pH 2.3. The line represents the linear least squares fit of eqn. 7 to the data.

As stated above, significant formation of the ternary complexes $Fe(L^8)(H_xL^y)^z$ requires an excess of aminohydroxamate ligand, and an increase in $[H^+]$ will tend to drive the equilibrium towards ternary complex dissociation. Both of these factors are exploited to dissociate the ternary complex in the receiving phase and stop the reformation of the host–guest complex and the subsequent back extraction of iron into the source phase. However, complete compartmentalization of the iron in the receiving phase in these experiments is not possible due to the lipophilicity of the $Fe(L^8)(OH_2)_2^+$ complex. The $Fe(L^8)(OH_2)_2^+$ complex has a pK_a of 6.36[32] and the neutral/deprotonated species will partition to a limited extent into the membrane phase[11] and diffuse back into the source phase. A similar iron transport system using a more hydrophilic tetradentate ligand, such as rhodotorulic acid or alcaligin,[11,35–44] could be used to completely compartmentalize the iron.

Conclusions

We have demonstrated the efficacy of a strategy for selective BLM transport of Fe(III) *via* ternary complex formation utilizing a hydrophilic bi-functional molecule, P~Q, with (i) a host functionality capable of coordinating to a vacant or labile coordination site on Fe(III) to form a hydrophilic ternary complex and (ii) a guest functionality capable of being recognized by a hydrophobic host. BLM transport is facilitated by ternary complex formation in the aqueous source phase, followed by molecular recognition *via* second coordination sphere host–guest complexation, diffusion of the resultant hydrophobic supramolecular assembly across the BLM and deposition of the ternary complex on the opposite side of the membrane (Fig. 2). Variations in the flux (Fig. 3) are consistent with a diffusion-controlled BLM flux model (eqn. 7, Fig. 4).

It has been demonstrated that there is no significant difference between the host–guest complexation with DC18C6 and a protonated amine that is either one or two carbons removed from the iron center. This suggests that the alkyl group linking the amine to the hydroxamic acid moiety in our bi-functional recognition agents does not sterically hinder the protonated amine in forming host–guest assemblies with crown ether hosts. It has been shown that the presence of a carboxylic acid adjacent to the protonated amine in the bi-functional recognition agent $[(H_3L^{glu})^+]$ inhibits the formation of host–guest assemblies, probably due to intramolecular H-bonding

These data suggest a biologically relevant mechanism for cell receptor recognition of tetradentate siderophore Fe(III) complexes utilizing a labile recognition agent. This may prove to be an important process for utilization of multiple siderophores by a single organism and may shed light on the evolutionary rationale for tetradentate siderophore synthesis (as opposed to hexadentate), in spite of disadvantageous concentration requirements for iron sequestration.[1,3] This approach may also be applicable to selective compartmentalization and removal of specific metal ions in environmental remediation.

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