Electrochemical Behavior of the Fe(III) Complexes of the Cyclic Hydroxamate Siderophores Alcaligin and Desferrioxamine E

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The redox behavior of Fe(III) complexes of the *cyclic* hydroxamate siderophores alcaligin and desferrioxamine E was investigated by cyclic voltammetry. The limiting, pH independent redox potential ($E_{1/2}$ vs NHE) is −446 mV for alcaligin above pH 9 and −477 mV for ferrioxamine E above pH 7.5. At lower pH values, the redox potential for both complexes shifts positive, with a loss of voltammetric reversibility which is interpreted to be the consequence of a secondary dissociation of Fe(II) from the reduced form of the complexes. These observations are of biological importance, since they suggest the possibility of a reductive mechanism in microbial cells which utilize these siderophores to acquire Fe. For comparison purposes, cyclic voltammograms were obtained for Fe(III) complexes with trihydroxamic acids of cyclic (ferrioxamine E) and linear (ferrioxamine B) structures, with dihydroxamic acids of cyclic (alcaligin) and linear (rhodotorulic and sebacic acids) structures, and with monohydroxamic acids (acetohydroxamic and *N*-methylacetohydroxamic acids) at identical conditions. The observed redox potentials allow us to estimate the overall stability constants for fully coordinated Fe(II) complexes as log β^{II}(Fe_2alcaligin$_3$) = 24.6 and log β^{II}(ferrioxamine E) = 12.1. A linear correlation between $E_{1/2}$ and pM was found, and the basis for this relationship is discussed in terms of structural (denticity and cyclic/acyclic) and electronic differences among the {alkyl-NOH-CO-alkyl} type of hydroxamic acid ligands studied.

Introduction

Siderophores are natural, low molecular weight, highly specific Fe-chelating agents that usually include either hydroxamate or catecholate functional groups.[1] Their role in microorganism metabolism is to acquire Fe from the environment, a task that involves three steps: solubilization (chelation) of environmental Fe(III) from its highly insoluble hydroxides; transport to and across the cell membrane; and deposition at the appropriate site within the cell.[1,2] Once released in the cell the Fe is involved in various metabolic pathways, often as Fe(II). Since siderophores exhibit a high and specific affinity for Fe(III) (log $\beta > 30$),[1] the in vivo mechanism for Fe release is of interest and the subject of much study. The stability of Fe(II) siderophores is considerably lower than Fe(III) siderophores, and they also tend to be kinetically labile with respect to Fe(II) dissociation. Consequently, reduction of the Fe(III) siderophore complex at the site of deposition is an attractive mechanistic possibility for Fe release.[1,3,4] At physiological conditions, many iron siderophore complexes have redox potentials out of reach for biological reducing agents such as NADPH, NADH, or $FADH_2$.[1] However, under mildly acidic conditions the redox potential of some iron−hydroxamate siderophores increase significantly, so that reduction by cell reducing agents may be thermodynamically favorable.[5−7]

Among the many natural hydroxamates identified as siderophores there is a distinctive structural class: the endocyclic hydroxamates. Ferrioxamine E (Figure 1) is a cyclic trihydroxamic acid that is produced by *Streptomyces pilosus* along with ferrioxamine B as a major product.[8] Several cyclic dihydroxamic acids have been identified as well. Alcaligin (Figure 1) is isolated from the heterotrophic bacterium *Alcaligenes denitrificans* KN3-1[9] and *Alcaligenes xylosoxidans*[10] and from the human respiratory pathogens *Bordetella pertussis* and *Bordetella bronchiseptica*.[11,12] Bisucaberin (22-atom ring dihydroxamic acid) is isolated from the marine bacterium *Alteromonas haloplanktis*,[13] while putrebactin (20-atom ring dihydroxamic acid)[14] is isolated from *Shewanella putrefaciens*, a bacterium

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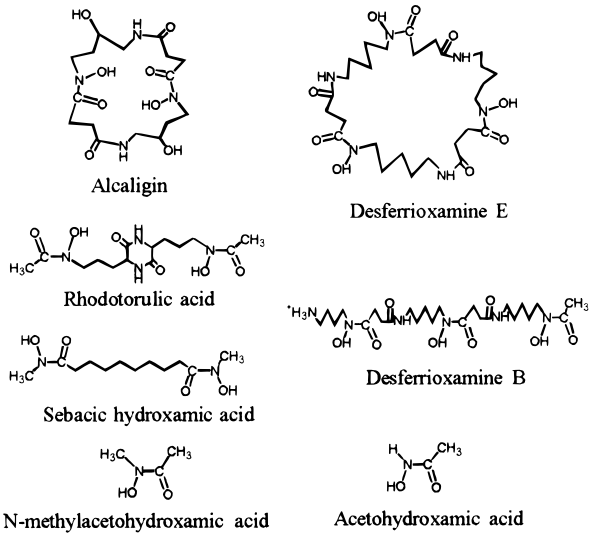

Figure 1. Hydroxamate siderophores and synthetic models.

found in natural waters, clinical specimens, oil pipelines, and spoiled food.[15]

The solution properties of the Fe(III) complexes of desferrioxamine E and alcaligin are known and they differ considerably when compared to the Fe(III) complexes of their linear analogues. Overall stability for both ferrioxamine E[16,17] and Fe_2(alcaligin)$_3$[18] is 100-fold higher than their corresponding linear siderophore counterparts ferrioxamine B[19,20] and Fe_2(rhodotorulic acid)$_3$.[21] The increased stability is assumed to be the result of a significant amount of structural pre-organization, which was nicely demonstrated in the work of Raymond et al. wherein the crystal structures of the Fe-free alcaligin ligand[10] and its Fe_2L_3 complex were compared.[18]

The electrochemical behavior of the Fe complexes of cyclic hydroxamate siderophores has not been investigated to date. Of particular interest are the redox potentials for the Fe(III) complexes of desferrioxamine E and alcaligin in relation to in vivo reducing agents, which may be relevant to the mechanism for cellular iron release. Also of interest is an assessment of the influence of endocyclic ligand structure on the observed Fe(III/II) redox potentials, as determined by comparing ferrioxamine E and alcaligin with Fe(III) complexes of analogous linear hydroxamic acid siderophores (Figure 1).

Experimental Section

Materials. Rhodotorulic acid (RA) was used as purchased from Aldrich. Desferrioxamine B (H_4DFB^+), methanesulfonate salt (Desferal), was purchased from Sigma, and its purity was established by complexation with Fe(III) in aqueous acidic solution.[22] Acetohydroxamic acid (AHA) was obtained from Aldrich and recrystallized twice from hot methanol. Ferric perchlorate stock solution was prepared from recrystallized solid ferric perchlorate hydrate and standardized for Fe^{3+} ion spectrophotometrically in strong acid.[23]

Alcaligin (AG) was purified from Fe-starved *B. bronchiseptica* culture supernatants by benzyl alcohol/ether extraction and recrystallization from hot ethanol.[11] Mass spectrometry and proton NMR spectroscopy were used to confirm the identity and purity of alcaligin preparations, which were found to consist of a single compound with spectral properties identical to the reported spectra of natural and synthetic alcaligin.[10]

Desferrioxamine E (H_3DFE) was kindly supplied by Prof. I. Fridovich and is produced by Ciba-Geigy as a side product in desferrioxamine B (Desferal) isolation from *S. pilosus.* It was further purified by recrystallization from hot methanol and its purity established by elemental analysis (anal. found (calcd): C, 53.99 (54.05); H, 8.05 (8.02); N, 13.99 (13.99)) and ^{1}H NMR spectroscopy (Varian Inova 400 MHz) in DMSO-d_6 (δ 1.15 (6H), 1.31 (6H), 1.45 (6H), 2.29 (6H), 2.59 (6H), 2.93 (6H), 3.41 (6H) 7.67 (3H), 9.53 (3H)).

***N,N′*-Dihydroxy-*N,N′*-dimethyldecanediamide, $[CH_3N(OH)C(O)]_2(CH_2)_8$, sebacic hydroxamic acid (SHA),** was prepared and characterized as described previously.[24] Anal. Found (calcd): C, 55.4 (55.5); H, 9.2 (9.3); N, 10.8 (10.7). ^{1}H NMR (GE QE 300 MHz, DMSO-d_6): δ 1.25 (8H), 1.48 (4H), 2.30 (4H), 3.07 (6H), 9.75 (2H).

***N*-Methylacetohydroxamic acid, $[CH_3N(OH)C(O)CH_3]$ (NMeAHA),** was prepared and characterized as described previously.[25] Anal. Found (calcd): C, 40.4 (40.5); H, 7.9 (8.0); N, 15.7 (15.8). ^{1}H NMR (GE QE 300 MHz, DMSO-d_6): δ 2.10 (3H), 3.30 (3H), 10.5 (1H).

Methods. Cyclic voltammetry measurements were performed using a PAR 362 analog scanning potentiostat with an analog *xy* chart recorder, or CH Instruments (computer-supported) potentiostat. A small volume (0.5 mL or 3 mL) cell with a three electrode setup was used: Ag/AgCl (reference), Pt wire (auxiliary), and glassy C or Au button (working electrode). The working electrode was carefully cleaned, polished (0.5 μm alumina), rinsed, and dried prior to each measurement. The potential of the reference electrode used relative to NHE was estimated by obtaining cyclic voltammograms of $Fe^{III/II}(CN)_6^{3-/4-}$ (+0.458 vs NHE in 0.5 M KCl)[26] as a calibrant. Redox potentials reported are determined from cylic voltammograms as $E_{1/2} = (E_{cathodic} + E_{anodic})/2$. Fe(III)−hydroxamate solutions were prepared by mixing the appropriate amount of an acidic $Fe(ClO_4)_3$ stock solution with an aqueous solution of ligand and $NaClO_4$. The pH was then adjusted by adding dilute NaOH or $HClO_4$ accompanied by vigorous stirring. All solutions were purged with ultrahigh-purity Ar (saturated with H_2O or 1:1 H_2O/MeOH, by vol) for 15 min prior to each voltammetric run. Confirmation of complex formation and ligand stoichiometry with Fe(III) was obtained from visible absorption spectra using an HP8451A diode array spectrophotometer.

Results

Solution Behavior of Fe(III)−Alcaligin and Fe(III)−Desferrioxamine E Complexes. The UV−visible absorption spectrum of an Fe(III) solution in the presence of excess alcaligin ligand at neutral pH shows a λ_{max} at 426 nm (ϵ = 2600 M^{-1} cm^{-1}/Fe), which is consistent with three hydroxamate moieties complexed to Fe(III).[22] This suggests a dimeric structure with the stoichiometry Fe_2L_3, as shown in the Fe_2(alcaligin)$_3$ crystal structure[18] and as reported for the solution behavior of rhodotorulic acid[21] and other synthetic[24] dihydroxamic acid complexes of Fe(III).

Ferrioxamine E solutions prepared at neutral pH showed identical UV−visible spectral characteristics as the alcaligin complex solution (λ_{max} at 426 nm, ϵ = 2600 M^{-1} cm^{-1}). The

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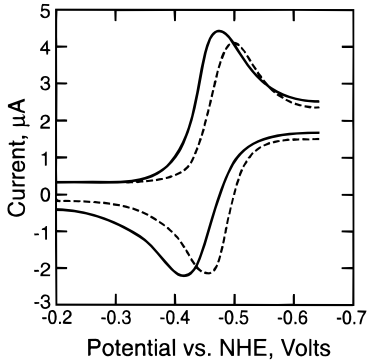

Figure 2. Cyclic voltammograms of Fe_2(alcaligin)$_3$ (solid line) and ferrioxamine E (dashed line). Conditions: $[Fe]_{tot} = 1$ mM, [alcaligin] = 20 mM or [desferrioxamine E] = 1.1 mM, 20 mM $NaHCO_3$, 20 mM NaH_2PO_4 and 50 mM "tris" buffer at pH 10.5 and 1 M $NaClO_4$; Au working electrode at 20 mV/s scan rate.

high stability of the fully coordinated complex makes it possible to prepare stable neutral aqueous solutions of FeDFE at submillimolar concentrations in the absence of excess ligand; this is fortunate since the H_3DFE ligand is only sparingly soluble in water.

Electrochemistry. Cyclic voltammetry was performed in order to investigate the redox chemistry of the Fe_2(alcaligin)$_3$ and ferrioxamine E complexes. For comparison purposes, cyclic voltammograms were obtained for Fe(III) complexes with trihydroxamic acid siderophores of cyclic (desferrioxamine E) and linear (desferrioxamine B) structures, with dihydroxamic acid siderophores of cyclic (alcaligin) and linear (rhodotorulic acid and sebacic hydroxamic acid) structures, and with monohydroxamic acid siderophore models (*N*-methylacetohydroxamic acid and acetohydroxamic acid), at identical conditions. Preliminary experiments demonstrated that the presence of excess ligand, and pH $\geq$ 7, are necessary prerequisites for obtaining reversible voltammograms and well-defined $E_{1/2}$ values for all of the hydroxamate complexes investigated, with the exception of ferrioxamine E and ferrioxamine B where 10% excess ligand was sufficient (Figure 2). At lower pH we observe a positive shift in $E_{1/2}$ and a loss of the reoxidation peak current. These effects are likely due to ligand dissociation events which occur in the reduced form of the complex.[6]

(a) Cyclic Voltammetry of Fe_2alcaligin$_3$ and Ferrioxamine E Relative to Other Iron-Hydroxamates. Cyclic voltammetry was performed for several Fe(III) hydroxamate complexes under identical conditions. For solubility reasons, this required the use of 1:1 CH_3OH/H_2O (v:v) solutions. A relatively high concentration of complex (5 mM) and only a 2-fold excess of ligand were used to ensure the presence of the hexacoordinate Fe complex as the only significant species in solution, while at the same time avoiding the possibility of forming higher monomeric species such as Fe(HL)$_3$ in the presence of larger excess ligand concentrations. The absence of buffer provided near-reversible behavior for all complexes examined, even at pH 7. However, in the presence of buffer (0.05 M phosphate), higher pH (9 and above) was needed to ensure the reversibility of the voltammograms. Results from the two separate sets of experiments (with and without buffer) are given in Table 1. These results clearly show the same trend in $E_{1/2}$ among several hydroxamates; the more stable Fe(III) complexes, as well as complexes with ligands of higher denticity, exhibit more negative $E_{1/2}$ values.

(b) pH-Dependent Cyclic Voltammetry of Iron−Hydroxamates of the {Alkyl-NOH-CO-alkyl} Type: Limiting Potentials. To more closely investigate the observed differences in redox potential among different hydroxamates and also to ensure that the differences observed are not a consequence of speciation effects caused by different complex stabilities in solution, pH dependent cyclic voltammetry was performed using Fe−hydroxamates of primary interest (those of {alkyl-NOH-CO-alkyl} type). The distribution of the various forms of complexed Fe(III) (e.g. FeL, FeL_2, etc.) varies with the ligand (L) at a fixed set of conditions, due to differences in overall and stepwise stability constants for the various complexes. We expect any complex where Fe is bound to less than six ligand donor atoms to have a more positive redox potential than a fully hexacoordinated complex. For example, in the case of acaligin, when the tetracoordinated Fe(III) complex ($FeL(H_2O)_2^+$) is the only species present in solution (pH 2 and an Fe/alcaligin ratio of 1:1), we observe an irreversible wave that is shifted 400 mV positive of the reduction wave of the hexacoordinated complex (Fe_2L_3). Thus, the observed trend in redox potentials shown in Table 1 might be solely due to the differences in the solution speciation of the different Fe complexes.

Table 1. Redox Potentials of Fe−Hydroxamate Complexes at Identical Concentration and pH Conditions

iron complex	$E_{1/2}$ mV[a] (NHE) pH 7 no buffer	E_{pp} mV[b]	i_a/i_c[c]	$E_{1/2}$ mV[d] (NHE) pH 10.5 buffered	E_{pp} mV[b]	i_a/i_c[c]
Fe(AHA)$_3$	−293	104	0.93	–	–	–
Fe_2(rhodotorulic acid)$_3$	−422	110	0.95	−423	69	0.94
Fe_2(SHA)$_3$	−434	89	0.92	−435	67	0.93
Fe_2(alcaligin)$_3$	−443	107	0.94	−447	98	0.95
ferrioxamine B	−482	82	0.96	−483	63	0.97
ferrioxamine E	−481	83	0.98	−478	71	0.97

[a] Conditions: $[Fe]_{tot}$ = 5 mM, [ligand] = 2-fold excess over stoichiometric requirement, 1 M $NaClO_4$, CH_3OH/H_2O 1/1 (v/v); glassy carbon electrode, scan rate 20 mV/s. Reproducibility ±2 mV. [b] Anodic−cathodic peak separation. [c] Ratio of anodic/cathodic peak currents. [d] Conditions: $[Fe]_{tot}$ = 2.5 mM, [ligand] = 2-fold excess over stoichiometric requirement, 0.05 M NaH_2PO_4, 0.05 M $Na_2B_4O_7$, 1 M $NaClO_4$, CH_3OH/H_2O 1/1 (v/v); Au electrode, scan rate 10 mV/s. Reproducibility ±2 mV.

It is possible to obtain a limiting, pH independent, redox potential at conditions where the Fe(III) and Fe(II) complexes are fully coordinated and no ligand exchange occurs during the time frame of the experiment. The necessary conditions are an excess of ligand and a sufficiently high pH to ensure full Fe coordination and no ligand exchange in the reduced form of the complex. A species distribution diagram for Fe(III) complexes of the {alkyl-NOH-CO-alkyl} type ligands was calculated using the same concentration conditions under which their limiting redox potentials were determined (Figure 3). These data illustrate that the limiting potentials of all complexes were determined under conditions where the hexacoordinated Fe(III) complex is the only Fe species in solution.

Figure 4 presents $E_{1/2}$ data as a function of pH obtained by cyclic voltammetry for ferrioxamine E and Fe_2(alcaligin)$_3$. Similar plots were obtained for Fe complexes with the linear siderophores (desferrioxamine B, rhodotorulic acid, and NMeA-HA). The limiting potential was taken as an average of the last several data points that showed a leveling-off behavior. Table 2 lists the $E_{1/2}$ values determined under such conditions.

It is noteworthy that the same trend in Fe(III/II) $E_{1/2}$ values with changes in hydroxamate siderophore ligand is observed when the limiting half-wave potentials are compared (under different conditions necessary to provide pH independence for the voltammograms; Table 2) and when half-wave potentials

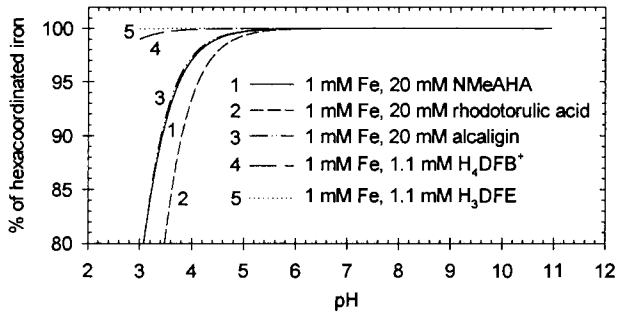

Figure 3. Percentage of Fe present as hexacoordinated Fe(III) complex with different hydroxamate ligands in aqueous solution as a function of pH. Simulation of the solution species distribution obtained by SPE program[29] utilizing overall stability constants β for Fe(III)−ligand complex species and protonation constants of ligands K_a. Concentrations of Fe(III) and ligands are as in limiting potential experiments (Table 2).

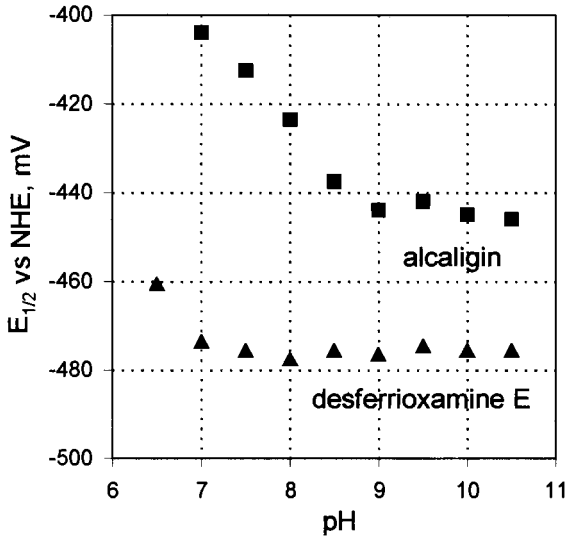

Figure 4. The pH dependence of the half-wave potentials ($E_{1/2}$) for Fe complexes of alcaligin and desferrioxamine E determined by cyclic voltammetry using a Au working electrode. Conditions are as given in Table 2.

are compared for all complexes under identical concentration conditions as described above (Table 1).

Discussion

The redox behavior of Fe complexes of the cyclic hydroxamates alcaligin and desferrioxamine E, and of their linear analogues (Figure 1), was investigated by cyclic voltammetry. At neutral and higher pH, fully coordinated Fe complexes of alcaligin and desferrioxamine E, as well as other {alkyl-NOH-CO-alkyl} type Fe−hydroxamate complexes, exhibit potentials too negative to be reduced by biological reducing agents (NADPH, NADH, or $FADH_2$). However, at lower pH a substantial positive shift in the redox potential, accompanied by a loss of reversibility, brings the redox potential within the reach of the reduction potentials of in vivo reducing agents. This behavior is observed for all Fe−hydroxamate complexes investigated in our laboratory and by others.[5,6] Nevertheless, in vitro experiments on the reduction of ferrioxamine B by an excess of NADPH, although of some success at pH 3.5 and in the presence of excess Fe, proved to be fruitless at pH 4.5.[6] A kinetic limitation has been proposed as an explanation for the absence of the reduction reaction.

The differences in redox potentials that we observe among Fe complexes of hydroxamates of the {alkyl-NOH-CO-alkyl} type are intriguing from a fundamental point of view. At the conditions where the limiting, pH independent redox potentials were determined, we may utilize a simple model that describes the electrochemical and chemical equilibria existing between fully coordinated Fe(III)− and Fe(II)−hydroxamate complexes and the uncomplexed aqua ions, $Fe(H_2O)_6^{3+}$ and $Fe(H_2O)_6^{2+}$, respectively. The model leads to eq 1. Once the standard redox

$$E^0_{\text{complex}} - E^0_{\text{aq}} = -59.15 \log(\beta^{\text{III}}/\beta^{\text{II}}) \qquad (1)$$

potential for $Fe(H_2O)_6^{3+}/Fe(H_2O)_6^{2+}$, $E^0_{\text{aq}} = +0.732$ (1 M $HClO_4$),[27] and the overall stability constant for the Fe(III) complex (β^{III}) is obtained from solution studies, eq 1 may be used to estimate the overall stability constant (β^{II}) for the fully coordinated Fe(II)−siderophore complex from measured E^0_{complex} (i.e., $E_{1/2}$) values. Table 2 lists the values of β^{II} calculated in this way for several Fe siderophore complexes of the {alkyl-NOH-CO-alkyl} type. One interpretation of eq 1 is that any expected change in $E^0_{\text{complex 1}}$ relative to $E^0_{\text{complex 2}}$ must come from a change in the ratio $\beta^{\text{III}}_{\text{complex 1}}/\beta^{\text{II}}_{\text{complex 1}}$ relative to the ratio $\beta^{\text{III}}_{\text{complex 2}}/\beta^{\text{II}}_{\text{complex 2}}$. For example, in order for the redox potential of an Fe complex to be shifted to a more negative potential, a ligand must increase the stability of the Fe(III) complex relative to the corresponding Fe(II) complex.

Comparing hydroxamate ligands of different denticity is complicated by the facts that their Fe complexes are of different stoichiometry and nuclearity and that their overall concentration stability constants have different units. Consequently, it is not surprising that we observe no simple relation when $E_{1/2}$ is plotted against log β^{III}. An alternative way to express the "complexing power" of ligands of different denticity on a common basis is through the concentration of the free Fe left uncomplexed in solution under a set of arbitrary conditions. Raymond and coworkers have established the pM value as the negative logarithm

Table 2. Limiting (pH Independent) Redox Potentials, Fe(III) and Fe(II) Complex Stability Constants, and pM Values

iron complex	$E_{1/2}$[a] mV (NHE) (limiting, high pH)	log β^{III} [b]	log β^{II} [c]	pM[d]
$Fe(NMeAHA)_3$	−348	29.4[e]	11.1	16.2
Fe_2(rhodotorulic acid)$_3$	−419	62.3 [f]	21.1 [f]	21.9
	−415[f]	(31.2) [g]	(10.6) [g]	
			23.3 [h]	
			(11.7) [g]	
Fe_2(alcaligin)$_3$	−446	64.6 [i]	22.6	23.0
		(32.3) [g]	(12.3) [g]	
ferrioxamine B	−483	30.6 [j]	10.0	26.6
ferrioxamine E	−477	32.5 [k]	12.1	27.7

[a] Average of $E_{1/2}$ values (±3 mV) obtained in pH region where $E_{1/2}$ no longer varies with pH. Conditions: [Fe(III)] = 1 mM, [ligand] = 20 mM for NMeAHA, rhodotorulic acid, and alcaligin, and 1.1 mM for desferrioxamine B and desferrioxamine E, 20 mM $NaHCO_3$, 20 mM NaH_2PO_4, and 50 mM "tris" buffer. Au working electrode, scan rate 20 mV/s. [b] Overall stability constant for formation of the Fe(III)−hexacoordinate complex; data taken from the literature. [c] Overall stability constant for formation of the Fe(II)−hexacoordinate complex calculated from eq 1. [d] $-\log[Fe(III)_{aq}]$ at pH 7.4 and $[Fe(III)]_{tot} = 10^{-6}$ M, $[ligand]_{tot} = 10^{-5}$ M calculated from literature stability constant data for the complexes and pK_a data for the ligands using the SPE program of Martell and Motekaitis.[29] [e] Reference 25. [f] Reference 21. [g] Per Fe values, calculated as $^1/_2$ log β. [h] This work. Calculated from eq 1 using $E^0(Fe_{aq}) = +732$ mV vs NHE, which was determined in 1 M perchlorate—the same conditions as in our experiments—rather than more commonly used value of +701 mV vs NHE (1 M chloride). [i] Reference 18. [j] Reference 19 and 20. [k] Reference 16 and 17.

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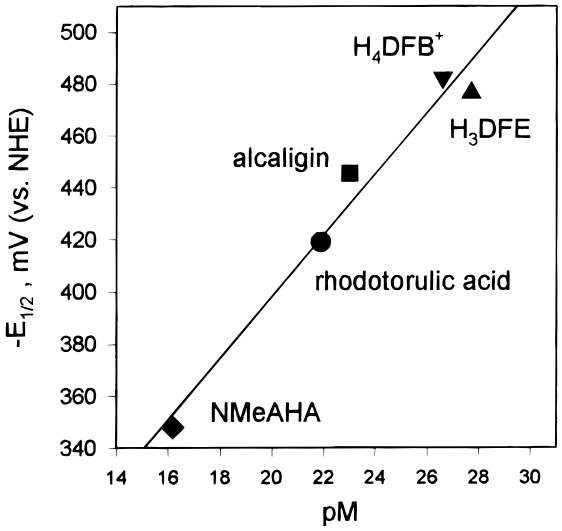

Figure 5. Limiting, pH independent redox potential ($E_{1/2}$; Table 2) plotted as a function of pM for Fe complexes of different hydroxamate ligands of the {alkyl-NOH-CO-alkyl} type.

of the free Fe^{3+} concentration in solution at pH 7.4, at total Fe(III) and ligand concentrations of 10^{-6} and 10^{-5} M, respectively.[28] We have calculated pM values for the complexes investigated using the SPE program of Martell and Motekaitis[29] which simulates the solution speciation based on the overall stability constants, β, of the Fe−hydroxamate complexes and protonation constants, pK_a, of the ligand. The values calculated here are in full agreement with pM values previously reported for ferrioxamine E, ferrioxamine B, and rhodotorulic acid.[30] When $E_{1/2}$ for all of the {alkyl-NOH-CO-alkyl} type siderophores investigated is plotted against their corresponding pM values,[31] a linear relationship is evident (Figure 5). This illustrates that as the stability of the complex increases (larger pM value), the redox potential shifts to more negative values in a predictable fashion. A similar correlation has been observed by others for Fe(III) complexes of ligands with catechol moieties (natural and synthetic proteins, simple catechols, and enterobactin).[32] In our work the focus is on Fe(III) complexes of linear and cyclic hydroxamate ligands with very similar electronic properties but different denticities. In that context, Figure 5 suggests that the redox potential of the complexes, being a direct function of their stability, is affected more by ligand denticity than by cyclization (closure) of the ligand backbone.

As the ligand denticity increases, the overall stability of the complex increases (higher β and pM values), and its redox potential becomes more negative. This is illustrated in Table 2 and Figure 5 for linear mono- (NMeAHA), di- (rhodotorulic acid), and trihydroxamate (desferrioxamine B) complexes. From eq 1 we can see that the change in redox potential between two complexes occurs because the ratio β^{III}/β^{II} (or pM^{III}/pM^{II} at neutral pH and above) for the two complexes changes. That is, there is an increasing preference of the ligand with higher denticity for Fe(III) over Fe(II). Why the 3+ state of the metal center is more stabilized than the 2+ state in going from one ligand to another is unclear. An inductive effect of the longer alkyl chains that have replaced the methyl groups of the ligands of lower denticity (Figure 1) may modify the donor properties of the O atoms of the hydroxamate moiety.[33] This may cause the ligand to exhibit a greater preference for the hard Fe(III) cation over the softer Fe(II) cation and thus ultimately shift the redox potentials to more negative values.

When a ligand has a closed (cyclic) structure, like alcaligin or desferrioxamine E (Figure 1), it can be viewed as being *structurally preorganized* for chelation. This may explain why ferrioxamine E is 100-fold more stable than its linear analogue ferrioxamine B (Table 2). However, ferrioxamine E and B $E_{1/2}$ values are the same! If we accept the concept that the closed cyclic ligand backbone keeps the hydroxamate moieties in positions predisposed to Fe binding, thus providing less opportunity for external trapping agents (other ligands, H^+) to attack either the metal center or the ligand functionalities, there is no apparent reason to believe that such preorganization of the ligand will not increase the stability of the Fe(II) complex by the same extent as in the case of Fe(III) complex. In this context, the absence of the effect of cyclization on the redox potential for the ferrioxamine E/ferrioxamine B pair is not unexpected.

Our attempts to plot $E_{1/2}$ values against log β^{III} produced a "quasilinear" correlation with a significant deviation of ferrioxamine B from the line. This raises a question of whether the β^{III} value for ferrioxamine B is anomalous due to its positively charged amine side chain, when compared to analogous uncharged siderophore complexes. However, log β^{III} values for the closest uncharged linear ferrioxamine B analogues (ferrioxamine D_1 (log $\beta^{III} = 30.76$)[1] and ferrioxamine D_2 (log $\beta^{III} = 31.40$)[1]) suggest that the protonated amine group has little effect on the stability of the Fe(III) complex.

In addition to structural preorganization, a *functional group predisposition* was reported by Raymond et al.[18] for cyclic alcaligin, where both hydroxamate groups in the Fe-free ligand are prepositioned for coordination to an Fe(III) center. As these authors have shown,[18] this situation results in an increase in the overall stability of the Fe_2(alcaligin)$_3$ complex as compared to its linear analogue, Fe_2(rhodotorulic acid)$_3$.[21] Unlike the case of the ferrioxamine E/ferrioxamine B pair, the difference in redox potential between Fe_2(alcaligin)$_3$ and Fe_2(rhodotorulic acid)$_3$ is significant (Tables 1 and 2, Figure 5). In the case of the alcaligin/rhodotorulic acid pair, it is the tetracoordinated complex $(FeL(H_2O)_2)^+$ rather than Fe_2L_3) that is affected by the predisposed cyclic structure of the alcaligin ligand. Thus, the effect of the preorganization only partially affects the *overall* stability of the hexacoordinated Fe_2L_3 complex. This opens the possibility that other effects may influence the stability of Fe(III) relative to Fe(II) complexes and thus influence the redox potential as well. Namely, in the alcaligin/rhodotorulic acid pair, the two methyl groups of rhodotorulic acid are replaced by longer alkyl chains in the alcaligin structure, whereby the electronic (inductive) effect may become an important factor which makes the ligands different in the way in which they

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(31) At neutral and higher pH, as can be seen from the species distribution diagram for all of the complexes investigated (Figure 3), the fully coordinated Fe species is predominant in solution. Under such conditions the pM value depends only on the overall stability constant (given that the pK_a values[16,19,21,25] of the ligands of {alkyl-NOH-CO-alkyl}type are essentially the same). Thus, at neutral and higher pH, the pM value may be viewed as representing a "normalized" overall stability of the fully coordinated Fe(III) complexes with ligands of different denticity.

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bind to the Fe(III)/Fe(II) pair and thus shifts the redox potentials of alcaligin and rhodotorulic acid Fe complexes apart. Furthermore, the alcaligin functional group predisposition for the Fe^{3+} ion may make coordination of the larger Fe^{2+} ion relatively less favorable and consequently shift $E_{1/2}$ for $Fe_2(alc)_3$ negative relative to $Fe_2(RA)_3$.

Conclusions

We have determined the limiting, pH independent redox potentials for fully coordinated Fe complexes of the cyclic hydroxamate siderophores alcaligin and desferrioxamine E as −446 mV and −477 mV vs NHE, respectively, as well as for several other synthetic and natural Fe-hydroxamate complexes. Based on these redox potentials and the overall stability constants for Fe(III) complexes (β^{III}), we estimate the overall stability constant for the Fe(II) iron complexes of alcaligin and desferrioxamine E (β^{II}), as $10^{24.6}$ and $10^{12.1}$, respectively. A linear relationship has been observed between two very important characteristics of Fe−siderophore complexes, redox potential, $E_{1/2}$, and overall complexing ability of the ligand, pM. It is a conclusion of our study that an increase in ligand denticity (connectivity between hydroxamate moieties) has a greater effect on $E_{1/2}$ than the closure (cyclization) of the ligand backbone of the same denticity (Figure 5).

It appears that microorganisms do not find the negative shift in the redox potential of ∼130 mV, in going from simple monodentate ligands (e.g. NMeAHA) to multidentate ligands (e.g. desferrioxamine B), as too high a price to pay in their efforts to synthesize multidentate ligands as better Fe sequestering agents. Our data suggest that perhaps the redox potentials of Fe complexes of natural hydroxamate siderophores are either *not* too negative for a reductive Fe-release mechanism to be operative (given the $E_{1/2}$ variation with pH and Fe/L ratio), or that a redox mechanism is coupled to some other *in vivo* process. Ligand hydrolysis, as another proposed pathway for removal of Fe from siderophores, would have the effect of decreasing the denticity of the ligand. Based on our results (e.g. Figure 5), this process should increase the reduction potential of the Fe(III) siderophore complex, making a reductive Fe release mechanism more favorable.

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