Coordination Chemistry of Microbial Iron Transport Compounds. 24. Characterization of Coprogen and Ferricrocin, Two Ferric Hydroxamate Siderophores[1]

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Abstract: The physical and thermodynamic properties of coprogen and ferricrocin, two hydroxamate siderophores produced by the fungus *Neurospora crassa*, have been examined. Formation constants determined by ethylenediaminetetraacetic acid (EDTA) competition reactions yielded the values log $K^* = 4.6$ and 3.3 and log $\beta_{110} \simeq 30.2$ and 30.4, respectively, where $K^* = ([FeL][H^+]^3)/([Fe^{3+}][H_3L])$ and $\beta_{110} = [FeL]/([Fe^{3+}][L^{3-}])$, showing that these siderophores possess affinities for ferric ion similar to other hydroxamate ligands. Redox potentials of −447 and −412 mV vs. NHE, respectively, were found. Circular dichroism measurements have demonstrated that coprogen adopts primarily a Δ absolute configuration about the iron, whereas ferricrocin exists as the Λ isomer.

Siderophores, microbial iron complexing agents, are remarkable because of their extremely high affinity for ferric ion.[4] The evolution of such molecules optimally designed for selective binding of iron(III) is a consequence of two factors: the necessity of this element for metabolism and the scarcity of iron(III) in a soluble form available to a cell. Siderophores function by removing iron from insoluble ferric hydroxides and presenting the metal as a soluble iron complex for transport into a cell. Release of iron is generally believed to occur following reduction of iron(III) to iron(II), and reductases have been reported for several hydroxamate siderophores.[5-7] In the case of enterobactin, partial degradation of the ligand by an esterase[8] is postulated as a key step in iron release because of its low redox potential,[9] although protonation has recently been suggested as an alternative, or accompanying, process.[1] In any case, the requirement of the hydrolysis step can be rationalized from the enormous stability of ferric enterobactin, which makes hazardous the continued presence of the enterobactin ligand within the cell.

Coprogen and ferricrocin are the two major siderophores produced by *Neurospora crassa*, although they have been obtained from various other fungi also. Under iron-deficient conditions, desferricoprogen is secreted into the growth medium.[10,11] In contrast, ferricrocin has been found in highest concentration in mycelial and conidial extracts.[12] Both of these siderophores act as germination factors for *N. crassa* conidiospores[12] and are transported by the same uptake system in the mycelia.[13] This system also transports the closely related compounds N-alkylated coprogen B's[14] and ferrichrysin but is inhibited by other ferricrocin analogues like ferrichrome A and ferrirubin. Ferrioxamines are not transported.[15]

The structure of desferricoprogen[10] in Figure 1 shows the molecule to be a modified trimer of N^5-hydroxy-N^5-(5-hydroxy-3-methyl-1-oxo-2-pentenyl)-L-ornithine. The three hydroxamic acid groups in their deprotonated form bind iron as an octahedral complex. Notable features of the ligand include the double bonds conjugated to the hydroxamate groups and the diketopiperazine ring, which also occurs in rhodotorulic acid (RA), a bis(hydroxamate) that forms binuclear iron complexes of the type $Fe_2(RA)_3$,[16] and in tris(ornicin), a recently discovered siderophore related to desferricoprogen.[17] Desferriferricrocin[18] also contains three hydroxamic acid groups, but they are linked instead by a cyclic hexapeptide that is basically [-(Orn)$_3$-Gly-Ser-Gly-]. There are many siderophores closely related to ferricrocin that differ only in some of the side chains, a few of which are shown in Figure 2. As expected, their chemical and biological properties are similar, though not identical.

Because of their high affinity for iron(III), siderophores or siderophore analogues are potentially useful wherever the need to chelate iron exists. One of the tris(hydroxamate) compounds, desferriferrioxamine B, has found extensive medical use in conjunction with treatment of thalassemias,[19] where excess iron must be removed from patients. This siderophore is now the drug of choice, although certain problems associated with its use have resulted in extensive efforts to obtain a better substitute; the search continues for iron(III) chelating agents of both natural and synthetic ligands in order to screen them for possible use as therapeutic agents. This paper describes the chemical properties of coprogen and ferricrocin in solution. The information is used to evaluate the iron chelating capabilities of the ligands and has implications for transport and iron release processes.

Experimental Section

Chemical analyses were performed by the Microanalytical Laboratory at the University of California, Berkeley.

The fast atom bombardment (FAB) mass spectroscopy was performed by the Space Science Laboratory at the University of California, Berkeley, and was recorded on a Kratos MS450, 40-na Xe at 8 kV.

A Jasco J-20 spectropolarimeter was used to obtain circular dichroism spectra, and all visible spectra were obtained with a Hewlett-Packard

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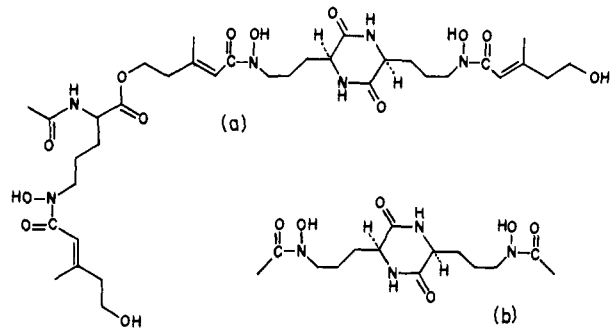
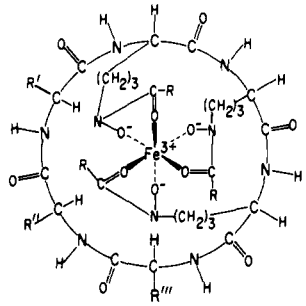


Figure 1. Structures of (a) desferricoprogen and (b) rhodotorulic acid.



Siderochrome	R′	R″	R‴	R
Ferrichrome	H	H	H	CH_3
Ferrichrysin	CH_2OH	CH_2OH	"	"
Ferricrocin	H	"	"	"
Ferrichrome A	CH_2OH	CH_2OH	"	$-CH=C(CH_3)-CH_2CO_2H$(*trans*)

Figure 2. Structures of ferricrocin and other ferrichrome-type siderophores.

8450A UV–VIS spectrophotometer.

Electrochemical Measurements. Electrochemical data were obtained through cyclic voltammetry at a hanging mercury drop working electrode referenced to the saturated calomel electrode (SCE). Linear voltage ramps were generated by a Princeton Applied Research Corporation Universal Programmer (Model 175)–Potentiostat (Model 173) system. All solutions were approximately 10^{-3} M in siderophore, containing 1 M KCl and 0.1 M Na/K phosphate buffer at pH 8.0.

Spectrophotometric Titrations. Spectrophotometric titrations of tris(hydroxamate) to bis(hydroxamate) equilibria of coprogen and ferricrocin were performed from pH 5 to 1. Solutions containing ~0.5 mM siderophore at 0.1 M KNO_3 and 25 °C were titrated with small amounts of ~0.1 M HNO_3, and the change in absorbance was monitored.

Potentiometric Titrations. Desferricoprogen (~0.5 mM) was titrated potentiometrically at 25.0 °C in 0.1 M KNO_3 with standardized, carbonate-free KOH.[20] Titrations of the free ligand took 45 min to 1 h. Ferric ion was added at the end of each titration, and a visible spectrum was taken to confirm that the ester linkage of the free ligand was not significantly hydrolyzed during the course of the titration.

Protonation constants were calculated by a weighted nonlinear least-squares refinement in which the log β's were varied to minimize the sum of the differences between the observed and calculated pH at each point in the titration curve.[21]

Spectrophotometric Competitions. Competition solutions were prepared containing 0.2–0.3 mM coprogen or ferricrocin, 0.5–100 equiv of EDTA (introduced as the disodium salt), and 0.1 M KNO_3 to control ionic strength. After the pH was adjusted to 5–7 and the solutions stood overnight, the pH was rechecked and the solutions were diluted to volume. Although no visible change was seen after 1 day, solutions were allowed to stand for 2 weeks at ~25 °C to insure attainment of equilibrium before absorbance maxima and pH were measured. Nine replicates with different initial siderophore and EDTA conditions were used for ferricrocin; 18 replicates were used for the EDTA competitions with coprogen.

Isolation, Purification, and Characterization of Coprogen and Ferricrocin. For production of desferricoprogen, a wild-type strain of *Neurospora crassa* 74A was grown in a chemically defined medium containing no additional iron. The medium contained the following additions per liter of distilled water: L-asparagine, 5 g; $K_2HPO_4 \cdot 3H_2O$, 1 g; $MgSO_4 \cdot 7H_2O$, 1 g; $CaCl_2 \cdot 2H_2O$, 0.5 g; $ZnSO_4 \cdot 7H_2O$, 20 mg; biotin, 10 μg; and glucose, 20 g (autoclaved separately). Thirty liters of medium were inoculated with a spore suspension (2-mL packed spores), which was obtained from agar cultures containing yeast extract, 4 g; malt extract, 10 g; and glucose, 4 g/L. Fermentation was performed at 27 °C under aeration for 5 days. After $FeCl_3 \cdot 6H_2O$ (aqueous solution 10^{-3} M) was added to the culture filtrate containing desferricoprogen, the resulting coprogen was adsorbed to Servachrome XAD-2 (200–250 μm) (Serva, Heidelberg) and washed with three volumes of distilled water. Coprogen was desorbed by the addition of one volume acetone/water (1:1) and concentrated under vacuum. The residual aqueous solution was lyophilized to yield ca. 6 g of crude coprogen.

The crude coprogen was dissolved in distilled water and passed twice through a column of carboxymethylcellulose (Servacel CM-32, Serva, Heidelberg) to adsorb coprogen B and other positively charged compounds. The effluent was concentrated to dryness and dissolved in pure methanol. After removing denatured proteins, the methanolic solution was passed through a column (8 × 100 cm) of Sephadex LH 20 (Deutsche Pharmacia, Freiburg). Three bands were observed, from which the main (middle) component was collected, concentrated, and lyophilized, yielding a highly purified material.

The purity of coprogen was confirmed by TLC, elemental analysis, and FAB–mass spectroscopy. The TLC was performed by using silica gel (Silica Gel 60, Merck, Darmstadt) and chloroform/methanol/water (65:25:4) as solvent system: coprogen R_f 0.4–0.45 (higher amounts increase the R_f value by 0.05). Anal. Calcd for $C_{35}H_{53}N_6O_{13}Fe \cdot 2H_2O$: C, 48.95; H, 6.76; N, 9.79%. Found: C, 48.61; H, 6.40; N, 9.53%. The $[M + 1]^+$ peak of the FAB mass spectrum was found at 822.46.

Purified coprogen (500 mg) was dissolved in 30 mL of water to prepare desferricoprogen. To this solution was added 10 mL of a methanolic solution of 8-hydroxyquinoline (oxine, 500 mg), and it was stirred overnight at room temperature. The black iron(III) oxinate was sedimented by centrifugation, and the clear supernatant was extracted five times with 20 mL of chloroform. The resulting aqueous solution was concentrated to dryness, dissolved in distilled water, and lyophilized, yielding ca. 350 mg of desferricoprogen. Anal. Calcd for $C_{35}H_{56}O_{13}N_6 \cdot 1.5H_2O$: C, 53.28; H, 7.15; N, 10.50%. Found: C, 52.95; H, 7.23; N, 10.59%.

Ferricrocin was isolated from the culture fluids of *Aspergillus viridi-nutans* Ducker and Thrower (CBS 127.65), obtained from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, by using the same cultivation medium, fermentation conditions, and purification steps as described for coprogen. A final purification on Bio-Gel P-2 (200–400 mesh) (Bio-Rad Laboratories, Munich) was included. The purity of ferricrocin was confirmed by TLC using silica plates and chloroform/methanol/water (65:25:4) as solvent system; ferricrocin R_f 0.55. Anal. Calcd for $C_{28}H_{44}N_9O_{13}Fe \cdot CH_3OH \cdot 1.5H_2O$: C, 41.99; H, 6.20; N, 15.19%. Found: C, 41.86; H, 6.20; N, 14.76. The $[M + 1]^+$ peak of the FAB mass spectrum was found at 769.1.

Results and Discussion

Visible Spectra. Absorbance maxima and extinction coefficients for coprogen[10] and ferricrocin[22] have been reported previously, but the λ_{max} values of 440 nm (ϵ = 2950) and 430 nm (ϵ = 2630), respectively, were somewhat different from ours. Since the method for determining the metal complex formation constants required an accurate value for the extinction coefficients, we decided to redetermine them for our observed λ_{max} values of 434 and 424 nm, respectively. Our values of ϵ_{434} = 2820 for coprogen and ϵ_{424} = 2460 for ferricrocin are in the same range as those for most of the other tris(hydroxamate) siderophores[16,22-26] (see Table I).

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(21) The weighted residual for each data point is $r_i = (1/\sigma_i)(pH_{obsd} - pH_{calcd})_i$. The derivatives $D_{ij} = (\partial r_i/\partial \log \beta_j)$ were computed numerically, and the shifts in β values, computed to minimize the sum of the squares of the residuals, were applied from the vector-matrix equation: $(\Delta \log \beta) = (D^TD)^{-1}D^Tr$. The weighting factor $1/\sigma_i$ was based on the estimated uncertainty in the pH reading at each point in the titration curve. This uncertainty has two components: the precision of the pH meter itself and the precision of titrant delivery (volume V_T). Thus the weight was calculated as

$$\sigma_i^2 = \sigma_{meter}^2 + \left(\frac{\partial pH}{\partial V_T}\right)_i^2 \sigma_{V_T}^2$$

where σ_{meter} = 0.003 pH unit, σ_{V_T} = 0.002 mL, and $\partial pH/\partial V_T$ is the slope of the titration curve at each point in the titration.

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Table I. Summary of Physical Properties[a]

metal complex	log β_{110}[b]	log K^*[b]	log K_{MHL}[b]	pM[c]	λ_{max}, nm (ϵ^d)	CD bands, nm ($\Delta\epsilon^d$) Δ	CD bands, nm ($\Delta\epsilon^d$) Λ	$\epsilon_{1/2}$, mV vs. SCE[e]
coprogen	30.2	4.6	0.50	27.5	434 (2820)	375 (+2.10) 475 (−1.26)		−691 (7)
ferricrocin	30.4[n]	3.3	0.53	26.5	434 (2460)		290 (−3.78) 360 (−1.62) 450 (+2.47)	−656 (7)
ferrichrome	29.1[f]	2.13	1.49[f]	25.2	425 (2895)[g]		~360 (−3.7)[h] ~465 (+2.4)	
ferrichrysin	30.0[f]	2.76		25.8	430 (3020)[i]			
ferrichrome A^{3-}					440 (3740)[g]		330 (−3.9)[j] 365 (−2.7) (sh) 465 (+3.2)	−690[k]
ferrioxamine B^+	30.5[f]	3.4	1.0[f]	26.6	428 (2800)[o]			−698[k]
Fe_2(rhodotorulate)$_3$				21.9	425 (2700)[l]	372 (+2.73)[m] 464 (−1.41)		−659[l]
Fe(benzohydroxamate)$_3$[j]					435 (4910)	350 (+2.3) 452 (−1.5)	350 (−2.8) 455 (+1.1)	

[a] All solutions are aqueous except for the benzohydroxamate complexes in acetone solution. [b] $\beta_{110} = [FeL^{3-n}]/[Fe^{3+}][L^{n-}]$, $K^* = [FeL^{3-n}][H^+]^3/[Fe^{3+}][H_3L^{3-n}]$, $K_{MHL} = [FeHL^{4-n}]/[H^+][FeL^{3-n}]$. [c] $pM = -\log [Fe(H_2O)_6^{3+}]$ when $[Fe]_T = 10^{-6}$ M, [L] = 10^{-5} M, pH 7.4. [d] Units are M^{-1} cm^{-1}. [e] Parentheses indicate uncertainty in the last digit. [f] Reference 35. [g] Reference 23. [h] Reference 28. [i] Reference 22. [j] Reference 25. [k] Reference 9. [l] Reference 24. [m] Reference 16. [n] Ligand protonation constants for ferricrocin were estimated from values in ref 35. [o] Reference 26.

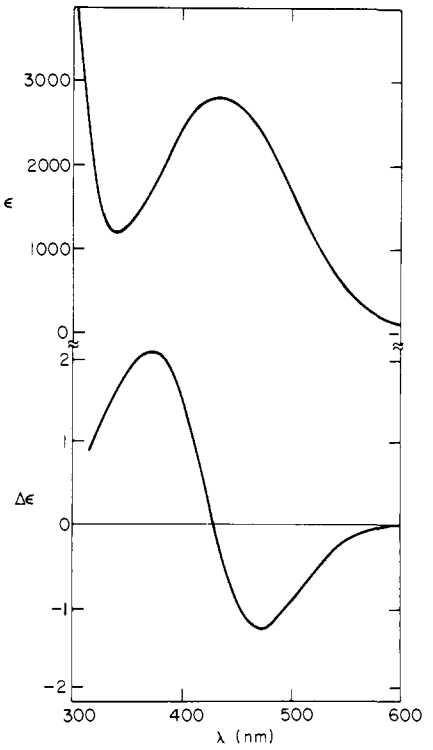

Figure 3. Electronic and circular dichroism spectra of coprogen.

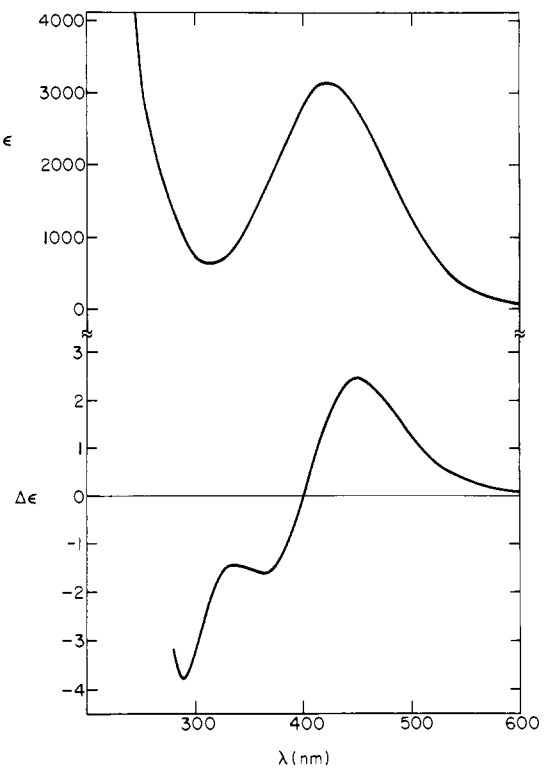

Figure 4. Electronic and circular dichroism spectra of ferricrocin.

Over the range of pH 3 to 8 no discernible change was seen in the visible spectrum of either compound, indicating that the complexes remain intact under these conditions. Unless otherwise specified, all experiments were carried out within the pH range 5–7.

Circular Dichroism Spectra. As a method of determining ligand chirality about a metal center in the absence of crystallographic data, circular dichroism (CD) is a valuable technique for the characterization of siderophores, since one can easily compare the spectra and hence chirality of similar molecules. By X-ray diffraction methods an unambiguous absolute configuration may be assigned to any one crystalline compound. This absolute configuration can then be related to the sign of the CD spectra. This has been done for ferrichrome A[25,27] and ferrichrome;[28] these siderophores adopt a Λ configuration and show a positive CD band near 465 nm in the region of strong visible absorbance. Both the Λ and Δ isomers of tris(benzohydroxamato)iron(III) have been isolated[25] and give CD spectra that are mirror images of each other. Circular dichroism data[16,25,28] are summarized in Table I.

Coprogen (Figure 3) displays a negative CD band at 475 nm with the same general spectral contours and intensities as Δ-tris(benzohydroxamato)iron(III), indicating that both complexes adopt the same chirality about the metal center. To date, coprogen

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and ferric *N,N',N''*-triacetylfusarinine[29] are the only two reported tris(hydroxamate) siderophore complexes that exist as the Δ isomers in solution. However, rhodotorulic acid, a bis(hydroxamate), forms a diferric complex with each iron in a Δ environment.[16] Since both desferricoprogen and rhodotorulic acid (H_2RA) contain a diketopiperizine ring with the same stereochemistry about the asymmetric carbons, it may be that this structural group plays a determining role in the metal complex configuration. Although the $[Fe(RA)]^+$ species that forms at pH 2 is monomeric and has quite a different structure from the Fe_2RA_3 dimer, the spectrum of $[Fe(RA)]^+$ is very suggestive that the Δ configuration also predominates in the monomeric complex.

Ferricrocin (Figure 4) shows a positive CD band at 450 nm and therefore must adopt a Λ configuration. The spectra are similar to those of the related compounds ferrichrome[28] and ferrichrome A,[25,27] which have been found to crystallize as their Λ isomers, as determined by X-ray diffraction methods. Crystallographic studies have shown that ferrichrysin[30] and ferric *N,N',N''*-triacetylfusarinine[29] also crystallize as the Λ isomers, although the latter apparently isomerizes to the Δ form in solution. A preliminary report on the crystal structure of ferricrocin[31] agrees with the Λ configuration assigned from the CD spectrum for ferricrocin in aqueous solution. Optical rotary dispersion measurements on ferricrocin, ferrichrome, ferrichrysin, and ferrichrome A are all similar.[18,32]

The CD spectra in Figures 3 and 4 appear more different than they actually are. Note that the two wavelength ranges are not identical. The ultraviolet cutoff for coprogen begins at lower energy, so that a band was not obtained corresponding to the 290-nm peak of ferricrocin. The differences in magnitude of $\Delta\epsilon$ may be due to two causes: a real difference in intrinsic $\Delta\epsilon$ or the presence of a mixture of Δ and Λ isomers for coprogen in which the Δ form predominates.

Transport of ferrichrome appears to be stereospecific in that the natural Λ-ferrichrome is recognized by the specific transport system of the cell membrane but not the synthetic Δ-enantioferrichrome.[33] Similarly, Δ-ferric enterobactin is recognized but not the synthetic Λ-ferric enantioenterobactin.[34] It has been suggested implicitly that, in general, the chirality of the metal center alone may be important for recognition by the membrane receptor. However, care must be taken in interpreting the role, if any, of the chiral metal center when it is only one of several asymmetric centers in the molecule or when different compounds are compared. For example, the coprogen transport system accepts ferricrocin, even though the chirality of the metal centers is different. Thus, the roles of the chiral metal centers vs. the ligand cannot be distinguished in the stereospecific recognition of Λ-ferrichrome vs. Δ-enantioferrichrome or Δ-ferric enterobactin vs. Λ-ferric enantioenterobactin. Unequivocal proof of the proposal that *metal* chirality is important for receptor recognition requires that kinetically inert Λ and Δ complexes of the *same* ligand be synthesized and that these complexes be subjected to the same transport tests.

Solution Thermodynamics. The ligand protonation constants for desferricoprogen were obtained by potentiometric titration at 25.0 °C in 0.1 M KNO_3. Titrations were performed quickly to avoid hydrolysis of the ester linkage, which is more labile in the free ligand. Addition of ferric ion to the titrated ligand solution and a spectrophotometric titration (vide infra) of the resulting complex confirmed the presence of the intact ligand. The refined constants[21] for desferricoprogen are

$$K_1 = \frac{[HL^{2-}]}{[H^+][L^{3-}]} \qquad \log K_1 = 9.16\ (5)$$

$$K_2 = \frac{[H_2L^-]}{[H^+][HL^{2-}]} \qquad \log K_2 = 8.86\ (5)$$

$$K_3 = \frac{[H_3L]}{[H^+][H_2L^-]} \qquad \log K_3 = 7.63\ (3)$$

Due to the limited amount of desferriferricrocin available to us, we have estimated the acid dissociation constants from those of other siderophores. An average of the protonation constants[35] of the closely related desferriferrichrome and desferriferrichrysin were used to estimate log K_1, log K_2, and log K_3 as 9.92, 9.01, and 8.14, respectively. It is interesting to note that the protons of desferricoprogen are considerably more acidic than those of other tris(hydroxamates). This is no doubt related to the aliphatic side chain, which contains a double bond conjugated to the hydroxamate group and stabilizes the anion by delocalization of the negative charge on the hydroxyl oxygen.

The extremely high stability of ferric tris(hydroxamate) complexes precludes direct measurement of the equilibrium of interest, which would yield the desired formation constant for the siderophore complex, β_{110}:[36]

$$Fe^{3+} + L^{3-} \rightleftharpoons FeL$$

$$\beta_{110}^{FeL} = \frac{[FeL]}{[Fe^{3+}][L^{3-}]}$$

One method of circumventing this problem is competition for the metal by another ligand.

The stability of ferric ion with ethylenediaminetetraacetic acid (EDTA) is of sufficient strength ($\beta_{110}^{FeEDTA} = 10^{25.0}$)[37] to compete effectively with the tris(hydroxamates) for ferric ion at intermediate pH values (5–7) if an excess of EDTA is used.

Knowing the equilibrium species concentrations permits a competition constant, K_{comp}, to be experimentally determined.

$$FeL + EDTA^{4-} \rightleftharpoons FeEDTA^- + L^{3-}$$

$$K_{comp} = \frac{[FeEDTA^-][L^{3-}]}{[FeL][EDTA^{4-}]}$$

By rearranging the equation and substituting the value from $\beta_{110}^{FeEDTA} = 10^{25.0}$, we obtain β_{110}^{FeL} for the hydroxamate complex:

$$\beta_{110}^{FeL} = \frac{[FeL]}{[Fe^{3+}][L^{3-}]} = \frac{\beta_{110}^{FeEDTA}}{K_{comp}}$$

The proton-dependent formation constant K^* is used if the values for the ligand protonation constants are not known

$$K^* = \frac{[FeL][H^+]^3}{[H_3L][Fe^{3+}]} = \frac{\beta_{110}^{FeL}}{\beta_{013}^{H_3L}}$$

where

$$\beta_{013}^{H_3L} = \frac{[H_3L]}{[L^{3-}][H^+]^3}$$

In these experiments the concentration of iron–siderophore complex for each solution, $[FeL]_T$, was calculated from the absorbance maximum and determined extinction coefficients. From mass balance relationships, total concentrations $[FeEDTA^-]_T$, $[EDTA]_T$, and $[L]_T$ were calculated, where the subscript T signifies inclusion of all protonated or hydrolyzed species. Since K_{comp} is defined specifically in terms of [FeL], $[FeEDTA^-]$, $[EDTA^{4-}]$, and $[L^{3-}]$, the total concentrations must be written in these terms.

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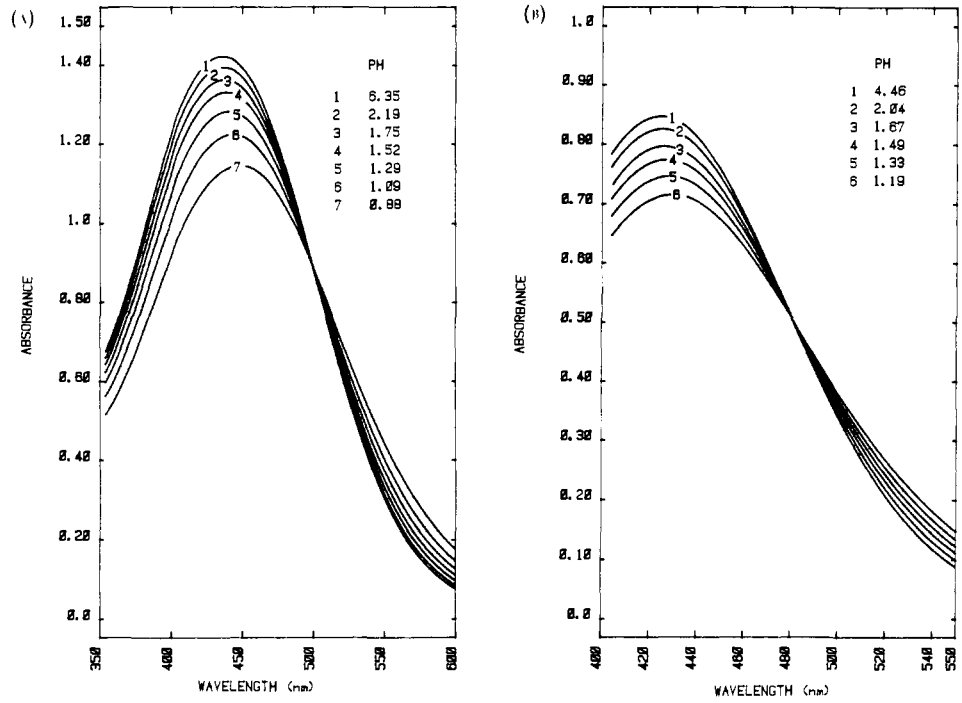

Figure 5. Spectrophotometric titration of (A) coprogen and (B) ferricrocin.

For example, EDTA exists in several different protonated forms at pH 5–7:

$$[EDTA]_T = [EDTA^{4-}] + [HEDTA^{3-}] + [H_2EDTA^{2-}]$$

Using the first and second protonation constants for $EDTA^{4-}$,[37] $K_1^{EDTA^{4-}} = [HEDTA^{3-}]/([H^+][EDTA^{4-}]) = 10^{10.17}$ and $K_2^{EDTA^{4-}} = [H_2EDTA^{2-}]/([H^+][HEDTA^{3-}]) = 10^{6.11}$, the above equation can be rearranged to give

$$[EDTA^{4-}] = \frac{[EDTA]_T}{1 + K_1^{EDTA^{4-}}[H^+] + K_1^{EDTA^{4-}}K_2^{EDTA^{4-}}[H^+]^2}$$

Through similar procedures, expressions for $[FeEDTA^-]$ and $[L^{3-}]$ were obtained by using $K_{ML}^{OH} = ([Fe(OH)EDTA^{2-}][H^+])/[FeEDTA^-] = 10^{-7.49}$ and ligand protonation constants. Under the pH conditions employed, coprogen and ferricrocin remain quantitatively unprotonated, hence $[FeL] = [FeL]_T$.

The formation constants ($\log \beta_{110}^{FeL}$ and $\log K^*$) for ferricrocin and coprogen are given in Table I. The numbers are comparable to those of related tris(hydroxamate) siderophores.[34]

Spectrophotometric titration of coprogen and ferricrocin by the method of Schwarzenbach[38] yields the metal complex protonation constants. Figure 5 shows the spectra of the tris(hydroxamate) to bis(hydroxamate) equilibria with an isobestic point at $\lambda = 498$ nm for coprogen and at 480 nm for ferricrocin. The metal complex protonation constant

$$K_{MHL} = \frac{[FeHL^+]}{[H^+][FeL]}$$

can be obtained from the slope of the Schwarzenbach plot:

$$A_{obsd} = \epsilon_{MHL}C_T + (1/K_{MHL})\frac{A_0 - A_{obsd}}{[H^+]}$$

where A_{obsd} is the absorbance at 434 nm (coprogen) and at 424 nm (ferricrocin) for various pH values, C_T is the total concentration of siderophore, ϵ_{MHL} is the extinction coefficient of the bis(hydroxamate), and A_0 is the initial absorbance at 434 nm (coprogen) or at 424 nm (ferricrocin) ($A_0 = \epsilon_{ML}C_T$ at high pH). The Schwarzenbach plots of the tris- to bis(hydroxamate) equilibria for coprogen and ferricrocin (Figure 6) give $\log K_{MHL} = 0.50$ and 0.53, respectively. These values are less than that observed for ferrioxamine B ($\log K_{MHL} = 1.0$)[35] and indicate that the tris(hydroxamate) complexes of coprogen and ferricrocin are stable down to lower pH values than are ferrioxamine or ferrichrome. Comparable values are listed in Table I.

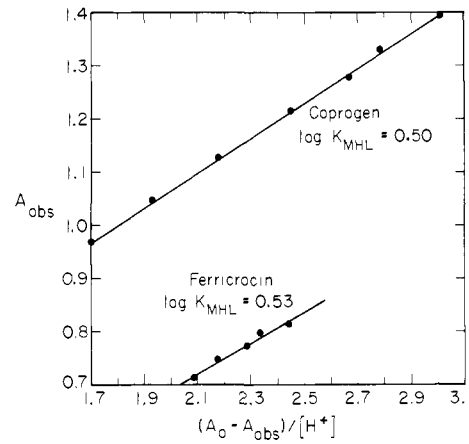

Figure 6. Schwarzenbach plots of coprogen and ferricrocin.

Although metal complex formation constants are important numbers in the evaluation of a ligand's metal affinity, β_{110}^{FeL} by itself is not the most appropriate measure under physiologically relevant conditions for two reasons: (1) At physiological pH, hydrogen ion is in sufficiently high concentration that it competes with iron for the desferrisiderophore trianion, such that the complexing form of the ligand L^{3-} is virtually nonexistent at pH 7. (2) Comparison with other ligands is difficult because the units of the formation constants differ, depending on the reaction stoichiometry. A pM scale has been introduced,[39] where pM = $-\log [Fe(H_2O)_6^{3+}]$ under conditions of $[Fe]_{total} = 10^{-6}$ M, $[L]_{total} = 10^{-5}$ M, and pH 7.4, to facilitate comparison under biologically reasonable conditions. Thus, a larger pM value reflects a higher affinity for iron under these conditions. An added advantage in cases where ligand protonation constants have been estimated and

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where the ligand is mostly protonated at pH 7.4 is that any uncertainties in the estimation have little effect on the pM calculation, since the ligand deprotonation reaction is insignificant.

Formation constants and pM values of coprogen and ferricrocin are given in Table I. The numbers are in accord with our expectations based on simple hydroxamates and comparisons with values of related tris(hydroxamate) siderophores.[35] From β_{110} and K^*, it is apparent that coprogen has a somewhat higher affinity for iron than any of the ferrichrome-type ligands. This observation may be explained (as can the greater acidity of the free ligand) by conjugation of the C=C bond with the hydroxamate group; this increases the electron density at the carbonyl oxygen through both inductive and resonance effects.

Formation constant data have been used for identifying which ligands could be useful in the treatment of iron-overload diseases. The most important criterion determining whether further tests should be done is that the ligand be capable of removing iron from the iron-transport protein, transferrin. Since coprogen and ferricrocin both have a significantly higher affinity for iron than transferrin (which has a pM of 23.6), both ligands are thermodynamically able to abstract iron from the protein. Whether they are kinetically able to do so is being examined currently.

Electrochemistry. Redox potentials for coprogen and ferricrocin were found to be −691 and −656 (±7) mV vs. SCE, respectively, or −447 and −412 mV vs. the normal hydrogen electrode (NHE). No significant shift was observed with different scan rates from 50 to 200 mV/s or with different concentrations of electroactive species. Peak-to-peak separations of 60–69 mV indicate that the electrochemical reactions are reversible or nearly reversible under our conditions. Comparison of these redox potentials to those of other hydroxamate siderophores shown in Table I indicate that all fall in the same range, approximately −0.40 to −0.45 V vs. NHE. These numbers have implications for the mechanism of release of iron(III) from coprogen and ferricrocin, since in the related ferrichrome system[5] it is believed that ferric ferrichrome is reduced to ferrous ferrichrome, followed by release of iron(II). A similar mechanism may be operating with coprogen and ferricrocin; an oxidoreductase capable of reducing both of these compounds has been reported for *N. crassa*.[6]

Summary

The electrochemical and thermodynamic properties of coprogen and ferricrocin have been found to be similar to those of other hydroxamate siderophores. Ferricrocin, as with other ferrichrome-type siderophore complexes, exists as the Λ isomer and possesses comparable formation constants and redox potentials. In contrast, coprogen adopts a Δ configuration in solution and has a somewhat higher affinity for iron(III) than other hydroxamates.

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Registry No. Desferricoprogen, 30315-65-2; coprogen, 31418-71-0; ferricrocin, 23086-46-6; ferrichrome, 15630-64-5; ferrichrysin, 18972-10-6; ferrioxamine B, 14836-73-8; EDTA, 60-00-4.

UV Photoelectron Spectra and Electronic Structure of $(\eta^5\text{-}C_5H_5)(CO)_2FeB_2H_5$. Comparison of the Fe–B Bonding with the Fe–C Bonding in $(CO)_4FeC_2H_4$

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Abstract: The valence level photoelectron spectrum of $(\eta^5\text{-}C_5H_5)(CO)_2FeB_2H_5$, I, has been measured in the gas phase with He I and Ne I radiation. The observed bands are assigned on the basis of model compound spectra, intensity changes with photon energy, and relative band areas. Molecular orbital calculations of the Fenske–Hall type on I as well as on $(CO)_4FeC_2H_4$, II, and the free ligands $B_2H_5^-$ and C_2H_4 corroborate the assignment of the spectrum as well as permit the detailed comparison of the Fe–B and Fe–C interactions in I and II. This comparison demonstrates that the B_2H_5 fragment in I can be considered as a $B_2H_4^{2-}$ side-on bound π ligand with a proton in the π lobe opposite the metal. The role of this proton is an active one in that contour plots show that the electron density distribution of the primary Fe–B interaction orbital of I is very different from that of the Fe–C interaction orbitals in II; namely, the Fe–B interaction is more aptly described as a closed three-center two-electron bond. Finally, extended Hückel calculations are used to demonstrate that the qualitative similarities and differences observed for $B_2H_5^-$ and C_2H_4 are apparent in the known higher homologues as well.

Despite the fact that boranes are considered to be "electron-deficient" compounds and exhibit the requisite electron acceptor properties, there are well-documented instances in which boranes act as electron donors.[1] Thus, for example, the electron-rich B–B bond in the pentagonal base of B_6H_{10} acts as an electron donor toward the $Fe(CO)_4$ fragment in the same manner as the C–C π bond in C_2H_4.[2] In recent years, more and more examples of borane fragments, isoelectronic with hydrocarbon π ligands, have

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