Coordination Isomers of Biological Iron Transport Compounds. II.[1] The Optical Isomers of Chromic Desferriferrichrome and Desferriferrichrysin

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Abstract: A number of microbial iron sequestering and transport agents (the siderochromes) are polyhydroxamic acids. In several of these agents, ferric ion has been replaced by chromic ion to induce kinetic inertness and thereby allow the possibility of isomer separation. The preparation and characterization of the chromic complexes of two siderochromes, desferriferrichrome and desferriferrichrysin, are reported. Although two coordination isomers are possible (Λ-cis and Δ-cis) for both complexes, both chromic complexes consist exclusively of the Λ-cis isomer. These results agree with X-ray crystallographic investigations which have shown that ferrichrysin and ferrichrome A both crystallize as only the Λ-cis isomer. Both chromic complexes have identical CD spectra. The chromic desferriferrichrome complex has CD maxima at 432 (−2.49), 571 (7.47), and 661 (−1.74) nm (Δε), while the chromic desferriferrichrysin complex has corresponding maxima at 431 (−2.48), 570 (7.31), and 659 (−1.86) nm. The chromic desferriferrichrome complex has visible absorption maxima at 583 (81) and 427 (70) nm (ε), while the chromic desferriferrichrysin complex has the same bands at 583 (80) and 427 (67) nm. The corresponding cobaltic complexes appear to be unstable because of gradual oxidation of the ligands by cobaltic ion.

The ferrichromes, shown in Figure 1, constitute an important class of microbial iron transport compounds,[3] in which the basic structural feature is a cyclic hexapeptide with the three hydroxamic acid linkages provided by a tripeptide of N^{δ}-acyl-N^{δ}-hydroxy-*l*-ornithine. Desferriferrichromes exhibit remarkable affinity for ferric ion, little affinity for other ions which differ in charge or size, and, in particular, little or no affinity for ferrous ion.[4,5] The first of these naturally occurring compounds to be isolated was ferrichrome, which Neilands found was produced by the smut fungus *Ustilago sphaerogena*.[6] It was found that ferrichrome displays potent growth factor activity for several microorganisms[7,8] and is produced only in high yield by *Ustilago* when the organism is grown under iron-deficient conditions.[9,10] These observations led Neilands to suggest that ferrichrome acts as a cellular transport cofactor.[11] The pathogenocity of certain infections is associated apparently with microbial iron transport, and several siderochromes are potent and broad spectrum antibiotics, while others are growth factors.[12,13] In all cases, however, the biological activity of these compounds centers on their unique ability to chelate iron and, following chelation, to take up the iron, *via* a cellular membrane transport system which is specific for the metal-bound hydroxamic acid.[14]

X-Ray crystallographic investigations have shown that ferrichrome A[15] and ferrichrysin[16] both crystallize as the Λ-cis isomer.[17] The observations that the ferric ion in siderochromes is ionic, high spin, and fairly rapidly exchangeable[18] are consistent with magnetic susceptibility,[19] Mössbauer,[20,21] and esr[21] data. Proton nmr data indicate that desferriferrichromes undergo a dramatic conformational change upon complexation with diamagnetic trivalent metal ions such as aluminum(III) and gallium(III).[22]

Many of the questions regarding the structure–function relationships of the siderochromes cannot be answered because of the kinetic lability of these high-spin ferric complexes. Surprisingly, the coordination chemistry of hydroxamic acids with metal ions other than ferric is largely unknown.[23] In a previous paper[1] we described the preparation of a simple model diastereoisomeric chromic complex, tris(*N*-methyl-*l*-menthoxyacethydroxamato)chromium(III), and the resolu-

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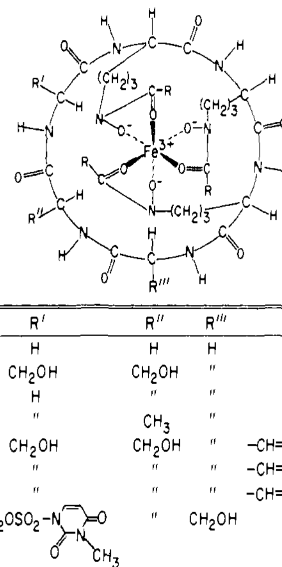
tion and characterization of its four geometrical and optical isomers: Λ-cis, Δ-cis, Λ-trans, and Δ-trans.

Although ferrichrome A and ferrichrysin both crystallize as the Λ-cis isomer, two diastereoisomeric coordination isomers are possible from an examination of molecular models: Λ-cis and Δ-cis. It is possible that both Λ-cis and Δ-cis isomers of these labile ferric complexes are in equilibrium in solution and that crystallization affords only the less soluble Λ-cis diastereoisomer. Replacement of ferric ion by chromic ion should induce kinetic inertness in these complexes and allow the separation of coordination isomers that exist in an equilibrium mixture. We report here the preparation and characterization of kinetically inert chromic complexes of desferriferrichrome and desferriferrichrysin. The corresponding cobaltic complexes appear to be unstable because of gradual oxidation of the ligands by cobaltic ion.



Siderochrome	R′	R″	R‴	R
Ferrichrome	H	H	H	CH_3
Ferrichrysin	CH_2OH	CH_2OH	"	"
Ferricrocin	H	"	"	"
Ferrichrome C	"	CH_3	"	"
Ferrichrome A	CH_2OH	CH_2OH	"	$-CH{=}C(CH_3){-}CH_2CO_2H$ (*trans*)
Ferrirhodin	"	"	"	$-CH{=}C(CH_3){-}CH_2CH_2OH$ (*cis*)
Ferrirubin	"	"	"	$-CH{=}C(CH_3){-}CH_2CH_2OH$ (*trans*)
Albomycin δ_1	$-CH_2OSO_2-N$(ring)$=O$, CH_3	"	CH_2OH	CH_3

Figure 1. Structure of the ferrichromes. The basic structural feature is a cyclic hexapeptide with the three hydroxamic acid linkages provided by a tripeptide of N^{δ}-acyl-N^{δ}-hydroxy-*l*-ornithine. The Λ-cis coordination isomer is shown in each case.

Experimental Section

Ultraviolet–visible spectra were measured with a Cary Model 118 spectrophotometer, and CD spectra were measured with a Jasco J-20 automatic recording spectropolarimeter. Chemical analyses were performed by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, Calif.

Materials. Reagent grade chemicals were used throughout. Ferrichrome, ferrichrysin, desferriferrichrome, and desferriferrichrysin were generously provided by Drs. M. Llinas and J. B. Neilands. Cobaltic hydroxide was purchased from K and K Chemical Co. Absolute ethanol was dried over Linde 3A molecular sieve. Gel filtration was performed on Bio-Gel P-2 (200–400 mesh, Bio-Rad Laboratories). Ion-exchange chromatography was performed on the cation exchange resin, AG 50W-X2 (200–400 mesh, sodium form, Bio-Rad Laboratories).

Thin-Layer Chromatography. Camag Kieselgel D-O silica gel was used for thin-layer and column chromatography, while Macherey Nagel cellulose powder MN 300 was used for thin-layer chromatography. Tlc on Kieselgel coated glass plates was performed on all of the metal complexes. Solvent systems were aqueous methanolic solutions, typically 30% H_2O–CH_3OH. Spots were detected visually or stained with iodine vapor.

Cobaltic Desferriferrichrome. A slurry of 790.6 mg (7.19 mmol) of cobaltic hydroxide and 500.0 mg (0.727 mmol) of desferriferrichrome in 25 ml of water was rapidly stirred at room temperature for 3 days. The slurry was suction filtered, and the green filtrate was concentrated to dryness *in vacuo*. The green solid was dissolved in a minimal amount of water and chromatographed on 12 g of Bio-Gel P-2 with water as the eluent on a glass column with o.d. of 14 mm. The major green fraction was concentrated to dryness *in vacuo*, and the residue was recrystallized from absolute methanol. Both the cobaltic complex and ferrichrome elute as single bands with identical R_f's on tlc with silica gel and cellulose powder with various solvent systems.[24]

Cobaltic Desferriferrichrysin. The procedure for cobaltic desferriferrichrome was followed using 73.5 mg (0.669 mmol) of cobaltic hydroxide and 50.0 mg (0.0669 mmol) of desferriferrichrysin in 10 ml of water. Both the cobaltic complex isolated from Bio-Gel P-2 filtration and ferrichrysin elute as single bands with identical R_f's on tlc with silica gel with various solvent systems.[24]

Chromic Desferriferrichrome. A solution of 120.0 mg (0.175 mmol) of desferriferrichrome and 38.7 mg (0.145 mmol) of $CrCl_3 \cdot 6H_2O$ in 180 ml of ethanol was refluxed under dry air ($CaCl_2$ drying tube) for 2 hr. The resulting blue solution was rapidly stirred overnight at room temperature. The blue slurry was then concentrated to dryness *in vacuo*, and the blue residue was washed with absolute methanol. The blue solid was chromatographed on 5 ml of sodium cation exchange resin on a glass column (o.d. of 11 mm) with water as the eluent. Gel filtration on Bio-Gel P-2 was then performed on the blue solid as described above. After gel filtration 67.8 mg (52.7%) was obtained. The blue solid, which was recrystallized from methanol, is hygroscopic. *Anal.* Calcd for $C_{27}H_{42}N_9O_{12}Cr \cdot 1.5H_2O$: C, 42.46; H, 5.94; N, 16.51; Cr, 6.81. Found: C, 42.8; H, 6.1; N, 16.5; Cr, 6.46. Both the chromic complex and ferrichrome elute as single bands with identical R_f's on tlc with silica gel and cellulose powder with various solvent systems.

Chromic Desferriferrichrysin. A solution of 200.0 mg (0.268 mmol) of desferriferrichrysin and 59.4 mg (0.223 mmol) of $CrCl_3 \cdot 6H_2O$ in 100 ml of ethanol was refluxed under dry air ($CaCl_2$ drying tube) for 1.5 hr. The resulting blue solution was rapidly stirred overnight at room temperature and then concentrated to dryness *in vacuo*. The blue residue was chromatographed first on a sodium cation exchange column and then on a Bio-Gel P-2 column as described above. Unreacted desferriferrichrysin was separated from the chromic complex by Kieselgel column chromatography as follows. Kieselgel (14.0 g) in 7.5% H_2O–CH_3OH was packed under nitrogen pressure in a glass column with o.d. of 19 mm. After topping the column with washed Monterey sand, a solution of 117 mg of complex in 0.3 ml of eluent was applied to the column and eluted under nitrogen pressure. A yellow band containing unreacted desferriferrichrysin eluted first, followed by a blue band containing the desired chromic complex. Since these two bands overlapped somewhat, the blue fraction was chromatographed again under the same conditions. Finally, a gel filtration on Bio-Gel P-2 was performed as described above. The isolated solid is hygroscopic. *Anal.* Calcd for $C_{29}H_{46}N_9O_{14}Cr \cdot 1.5H_2O$: C, 42.28; H, 6.00; N, 15.30; Cr, 6.31. Found: C, 42.6; H, 6.2; N, 15.4; Cr, 6.00. Both the chromic complex and ferrichrysin elute as single bands with identical R_f's on silica gel tlc, using various solvent systems.

Physical Measurements. Visible and CD spectra of both chromic complexes were determined in water solution at room temperature. The solution concentrations of chromium(III) were determined spectrophotometrically as $[CrO_4]^{2-}$ (ϵ_{372}^{max} 4815 l. mol^{-1} cm^{-1})[25] after oxidation of an aliquot of the chromium-containing solution with alkaline hydrogen peroxide. Excess hydrogen peroxide was removed by boiling the solution for 0.5 hr.

Results and Discussion

Visible absorption and CD data of the chromic complexes of desferriferrichrome and desferriferrichrysin are summarized in Table I. Both chromic complexes have visible spectra similar to those of the cis optical isomers of tris(N-methyl-*l*-menthoxyacethydroxamato)-chromium(III), which have spin-allowed d–d transitions

(24) Unsatisfactory elemental analyses for the cobaltic complex were obtained because of gradual oxidation of the ligand by the cobaltic ion. In addition, the cobaltic complex was observed to undergo aquation over a period of several hours in dilute acetic acid solutions and instantly in dilute mineral acid solutions. The internal redox reaction of the cobaltic complex and its kinetic lability are due to the relatively weak ligand field strength of these oxygen-donor ligands. The cobaltic complexes must be very close to the crossover between a low-spin and high-spin state.

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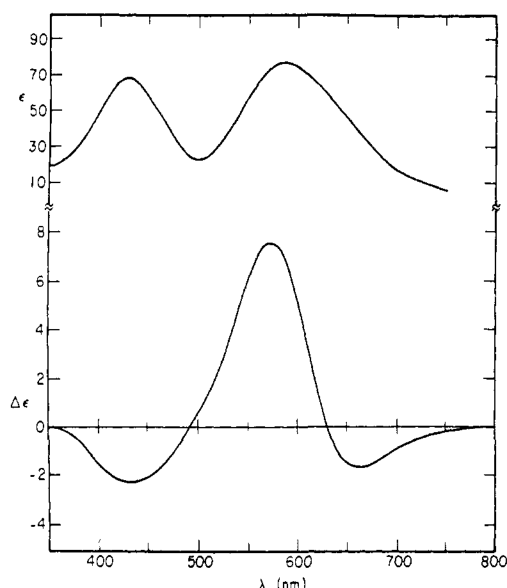

Figure 2. Visible absorption and CD spectra of chromic desferriferrichrome in aqueous solution. Chromic desferriferrichrysin has similar spectra.

Table I. Characterization of Chromic Complexes of Desferriferrichrome and Desferriferrichrysin

Assigned configuration[a]	Absorption max, nm (ϵ)[b]	CD_{max}, nm ($\Delta\epsilon$)[b]
Λ-Cis chromic desferriferrichrome	427 (70.1) 583 (81.0)	432 (−2.49) 571 (7.47) 661 (−1.74)
Λ-Cis chromic desferriferrichrysin	427 (66.9) 583 (79.7)	431 (−2.48) 570 (7.31) 659 (−1.86)

[a] See text for discussion of these assignments. [b] Data refer to the visible region only. Units are l. $mol^{-1}\,cm^{-1}$.

${}^4A_{2g} \rightarrow {}^4T_{1g}$ and ${}^4A_{2g} \rightarrow {}^4T_{2g}$ at 425 (66) and 592 (70) nm (ϵ), respectively.[1]

Buerer and Gulyas reported the visible absorption spectra of the chromic complex of desferriferrichrysin previously.[23] They reported maxima at 24.0 (1890) and 17.4 (1640) kK. Here the dipole strength, D, is given in parentheses after each maximum, in 10^{-40} cgs units. The dipole strength D can be determined experimentally from the area of an absorption band from eq 1[26] where

$$D = 91.8 \times 10^{-40} \int \frac{\epsilon}{\nu} d\nu \quad (1)$$

ϵ is the molar extinction coefficient at the frequency ν of the absorption band. If Gaussian absorption bands are assumed, D can be calculated from eq 2.[27] Con-

$$D = 97.7 \times 10^{-40} \frac{\epsilon_{max}\Delta\nu_{1/2}}{\nu_{max}} \quad (2)$$

version of our visible absorption data for chromic desferriferrichrysin using eq 2 gives 23.4 (1306) and 17.2 (1709) kK (D). We observe similar visible absorption maxima to those of Buerer and Gulyas but a significantly different dipole strength for the higher energy ${}^4A_{2g} \rightarrow {}^4T_{1g}$ transition.

The CD spectra of the chromic complexes of desferriferrichrome and desferriferrichrysin are identical; hence only the spectrum of the former complex is shown in Figure 2. Since the low energy, E_a, CD band at 570 nm is positive, both chromic complexes consist at least primarily of the Λ-cis optical isomer.[28] Since the CD spectra of both chromic complexes are virtually identical with that of the Λ-cis optical isomer of tris(*N*-methyl-*l*-menthoxyacethydroxamato)chromium(III), which has CD maxima at 429 (−2.82), 573 (7.83), and 670 (−1.85) nm ($\Delta\epsilon$),[1] both chromic complexes must consist exclusively of the Λ-cis optical isomer. Furthermore, both ferrichrome and ferrichrysin and their corresponding cobaltic and chromic complexes elute as single bands with identical R_f's on thin-layer chromatography with various sorbents and solvent systems. These results agree with X-ray crystallographic investigations which have shown that ferrichrysin[16] and ferrichrome A[15] both crystallize exclusively as the Λ-cis isomer.

Buerer and Gulyas also reported the CD maxima of the chromic complex of desferriferrichrysin in terms of the rotatory strength, R. The bands reported were 15.0 (−1.1), 17.65 (5.7), and 23.65 (−2.7) kK, where values of R in 10^{-40} cgs units are in parentheses.[23] The rotatory strength, R, can be obtained from the area of a CD band from eq 3[29] where ϵ_l and ϵ_r are the molar

$$R = 22.9 \times 10^{-40} \int \frac{\epsilon_l - \epsilon_r}{\nu} d\nu \quad (3)$$

extinction coefficients of left and right circularly polarized light, respectively. If Gaussian CD bands are assumed, R can be calculated from eq 4.[27] Con-

$$R = 24.5 \times 10^{-40} \frac{(\epsilon_l - \epsilon_r)_{max}\,\Delta\nu_{1/2}}{\nu_0} \quad (4)$$

version of our CD data for chromic desferriferrichrysin using eq 4 gives 15.2 (−4.2), 17.5 (22.6), and 23.2 (−11.2) kK (R). We observe similar CD maxima to those of Buerer and Gulyas but significantly greater CD rotatory strengths for all bands. Apparently Buerer and Gulyas' chromic complex of desferriferrichrysin was only about 25% pure.

In conclusion, visible absorption and CD data for the chromic complexes of desferriferrichrome and desferriferrichrysin agree well with the corresponding data for the previously reported model chromic complexes.[1] Although two optical coordination isomers are possible for both chromic complexes, we have found only one. Further studies of the synthesis and biological activities of these and related compounds are in progress.

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