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Complexation of Iron(III) by Cystinedihydroxamic Acid

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In acidic and neutral solutions, cystinedihydroxamic acid (H_2L^{2+}) binds ferric ion forming monomeric and dimeric complexes of 1:1, 2:2 and 2:3 metal to ligand stoichiometry. Comparison of the obtained equilibrium and spectral data for mono(cystinedihydroxamato)iron(III) with those of other hydroxamatoiron(III) complexes suggests the same mode of coordination. The Fe_2L_3 complex has been isolated and characterized by elemental analysis and IR spectra.

INTRODUCTION

Metal ion complexes of hydroxamic acids have drawn much attention due to their biological and clinical significance. They are biologically active as antibiotics, growth factors, tumour inhibitors, pigments and iron sequestering agents.[1–5] A natural linear trihydroxamic acid desferrioxamine B, is used in human medicine to remove excess iron in patients suffering from iron overload as a consequence of the treatment of Cooley's anemia, or acute iron poisoning. Removal of excess aluminium from the human body by the use of desferrioxamine B appears to be of importance in those patients who must undergo permanent hemodialysis.[6,7] Hydroxyurea, the simplest aminohydroxamic acid, shows antitumour activity and has been introduced into cancer therapy.[8] In an attempt to design iron(III) chelates as suitable

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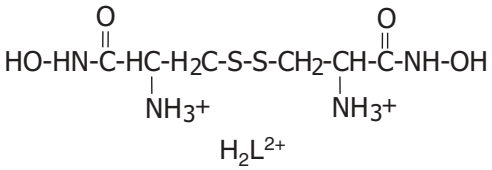
sources of iron in animal nutrition, iron(III) complexes with acet-, glycine- and histidinehydroxamic acid have been studied with promising results.[9,10]

Naturally occurring hydroxamic acids, such as desferrioxamine B, have typically three bidentate binding sites, making them hexadentate ligands. However, a small group of these compounds have only two hydroxamate binding sites. The most well-known example is rhodotorulic acid which forms a highly stable Fe(III) complex between pH 3 and 12.[11] A unique feature of dihydroxamic acids is their bifunctionality which gives them propensity to form dinuclear species of Fe_2L_3 stoichiometry.[12] Iron(III) dihydroxamate complexes have been proposed as contrast agents for NMR-imaging in medical diagnosis.[13] A series of dihydroxamic acids and their iron(III) complexes have been studied, but no ligand with sulphur atoms in the chain bridging two hydroxamate groups has been described to date. This paper deals with the properties of iron(III)-cystinedihydroxamato complexes.

RESULTS AND DISCUSSION

Acid-Base Properties of Cystinedihydroxamic Acid

In a common aqueous titration range, protonated cystinedihydroxamic acid could liberate four protons, two from protonated amino groups (NH_3^+), and another two from the OH groups of the hydroxamic moieties.



A potentiometric titration of the completely protonated ligand with potassium hydroxide revealed that four protons were titrated per ligand molecule. The obtained ionization constants are given in Table I. Comparison of these data with those for a series of dihydroxamic acids suggests that pK_{a_3} *and* pK_{a_4} may be attributed to the two hydroxamate groups.[14] The observed differences between the two pK_a values for the two hydroxamic acid groups as well as for the two amino groups are larger than 0.6 units. This is not consistent with the equivalent non-interacting site model which predicts a difference of 0.602 for two protonation sites that have the same intrinsic acidity, *e.g.* equal microscopic pK_a values, and are sufficiently removed from each other as to be completely non-reacting. This difference arises from statistical effects, since the second deprotonation step can only occur from a molecule that has already lost one proton. The obtained difference can be explained by the intramolecular hydrogen bonding throughout the cystinedihydoxamic acid molecule which brings two hydroxamate groups into close proximity, the behaviour of each of them becoming dependent on the other.

TABLE I

Equilibrium and spectral data for the cistinedihydroxamate acid and its iron(III) complexes, t = 25 °C, I = 0.1 M (KNO_3)

Specification	Cistinedihydroxamic acid		
Acidic constants	pKa_1 (-NH_3^+)	5.36 (0.05)	
	pKa_2 (-NH_3^+)	6.52 (0.03)	
	pKa_3 (-NHOH)	8.60 (0.02)	
	pKa_4 (-NHOH)	9.63 (0.02)	
Complex formation constants	log β ($FeHL^{4+}$)	18.93 (0.02)	19.0*
	log β ($Fe_2L_2^{6+}$)	33.91 (0.13)	
	log β ($Fe_2L_3^{6+}$)	46.44 (0.10)	
Molar absorptivities	$e \times 10^3$ $M^{-1}cm^{-1}$ ($FeHL^{4+}$)	1.96	1.70*
	$e \times 10^3$ $M^{-1}cm^{-1}$ ($Fe_2L_2^{6+}$)	2.62	
	$e \times 10^3$ $M^{-1}cm^{-1}$ ($Fe_2L_3^{6+}$)	3.41	

* at ionic strength of 1.0 M (KNO_3)

Formation of Mono(cystinedihydroxamato)iron(III) Complex

When an acidic aqueous solution of iron(III) is mixed with cystinedihydroxamic acid, a violet-red coloured complex is formed. The visible spectra of this complex, as a function of pH and iron(III) concentrations, are shown in insets of Figures 1 and 2, respectively.

The first step in the overall cystinedihydroxamato-iron(III) complexes formation is expected to be the formation of the mono(cystinedihydroxamato)iron(III) species. Coordination of the second ligand molecule could be avoided by keeping Fe(III) in excess over the hydroxamic acid in solutions. However, the two hydroxamate groups of H_2L^{2+} allow the possibility of formation of a bimetallic species such as Fe_2L^{6+}. Figure 2 shows the ratio of the total iron(III) concentration and the absorbance plotted *vs*. the total iron(III) concentration. The absorption data were collected at λ_{max} = 482 nm, where hydrated iron(III) ion does not exhibit significant absorption. The obtained linear relationship suggests that only one complex exists under these experimental conditions.[15] Hill's plot with a slope of about 0.9, shown in Figure 1, suggests that one H^+ ion is released per one H_2L^{2+} in the complexation reaction in the pH = 0.4–1.9 range. This means that equation (1) appropriately describes the equilibrium. The spectral data analysis confirmed the existence of only one complex under such experimental conditions, with 1:1 stoichiometry and one proton released in the complexation. The fact that λ_{max} = 482 nm is not changed in Figures 1 and 2 also confirms this findings. All these results suggest that the Fe_2L^{6+} complex has not been formed. This is in agreement with the expected electrostatic repulsion be-

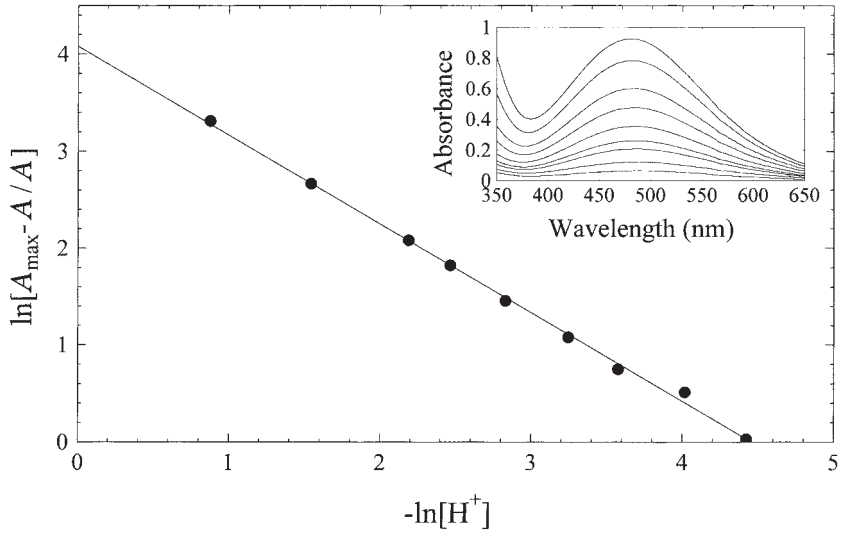


Figure 1. Determination of the number of hydrogen ions involved in the equilibrium (1). Molar absorptivity used for the calculation A_{max} at 482 nm is obtained from the plot depicted in the inset of Figure 1. Inset: Solution spectra of the iron(III) complex with cystinedihydroxamic acid at various pH. $[Fe^{3+}]_{tot} = 5 \times 10^{-3}$, $[CYS]_{tot} = 1 \times 10^{-3}$, I = 1.0 M (KNO_3) T = 25 °C, $[H^+]$ = 0.012–0.417.

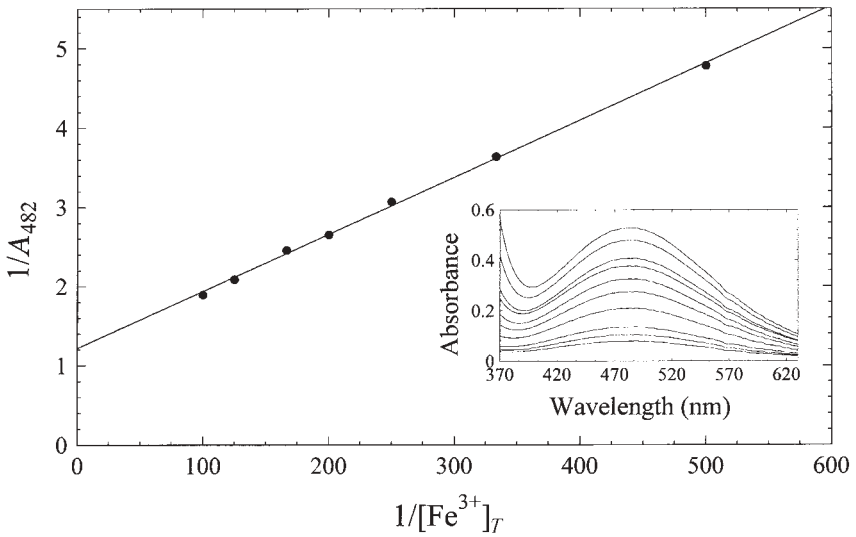


Figure 2. The ratio of the total iron(III) concentration to the absorbance at 482 nm *vs.* total iron(III) concentration. Inset: Solution spectra of the iron(III) complex with cystinedihydroxamic acid at various $[Fe^{3+}]_{tot}$. $[H^+]$ = 0.022, $[CYS]_{tot} = 5 \times 10^{-4}$, I = 1.0 M (KNO_3), T = 25 °C, $[Fe^{3+}]_{tot} = 5 \times 10^{-4} - 1 \times 10^{-2}$.

tween the positively charged two iron centres bridged by the coordinated cystinedihydroxamic acid, as proposed for analogous dinuclear complexes of iron(III) and some aliphatic dihydroxamic acids.[16]

$$H_2L^{2+} + Fe^{3+} \rightleftarrows FeHL^{4+} + H^+ \quad (1)$$

A general rule has been accepted[17] according to which the molar absorptivity of an iron(III) hydroxamate complex is about $1000n$ M^{-1} cm^{-1}, n being the number of hydroxamate groups bonded per iron(III) ion. The obtained molar absorptivity of 1700 M^{-1} cm^{-1} is higher than usual for monohydroxamato iron(III). This may be explained by the existence of positive charges on the complex (NH_3^+), which increases the degree of solvation and thereby molar absorptivity. The difference between equilibrium constants for the mono(cystinedihydroxomato) iron(III) (log K = 0.70) and the analogous iron complex of the ligand with six atoms in the aliphatic chain between the two hydroxamate groups (log K = 1.79) may be reasonably rationalized taking into account the positive charges on the ligand.[16] Our preliminary kinetic results indicate that the rate constant for the reaction between H_2L^{2+} and $FeOH^{2+}$ ($k = 8.75 \times 10^3$ M^{-1} s^{-1}) is of the same magnitude as that obtained for numerous other hydroxamic acids, *i.e.* monohydroxamic acids,[18–20] dihydroxamic acids,[16] and desferrioxamine B.[21,22] Therefore, the observed lability of $FeHL^{4+}$ is a result of the ease with which the ligand dissociates from the complex due to the high positive charge.

Formation of Bimetallic Iron(III) Cystinedihydoxamic Acid Complexes

In the solutions of cystinedihydroxamic acid in excess over iron(III), depending on the hydrogen ion concentration, formation of higher complex species takes place. However, very low solubility of the complexes formed at pH > 3 and at high ionic strength makes the concentration range for the stability constants study too narrow. In order to extend this range, we ran experiments at an as low as possible ionic strength. Figure 3 shows the spectral data acquired at constant ionic strength maintained by 0.1 M KNO_3, which allowed experiments to be performed at pH up to 5. Treatment of these spectra showed that a satisfactory fit was obtained only for the complexes of $FeHL^{4+}$, $Fe_2L_2^{6+}$ and $Fe_2L_3^{6+}$ stoichiometry. As shown in Figure 4, the calculated absorbancies are in good agreement with experimental data points. Distribution of the proposed complex species in the pH range 0.5–5 is shown in Figure 5. As shown in Table I, formation constants for the $Fe_2L_3^{6+}$ (log β = 46.4) complex are much lower than for rhodotorulic acid (log β = 62.2).[11] The obtained difference may be reasonably rationalized in terms of a high positive charge of $Fe_2L_3^{6+}$ due to the protonation of amino groups in the pH range studied.

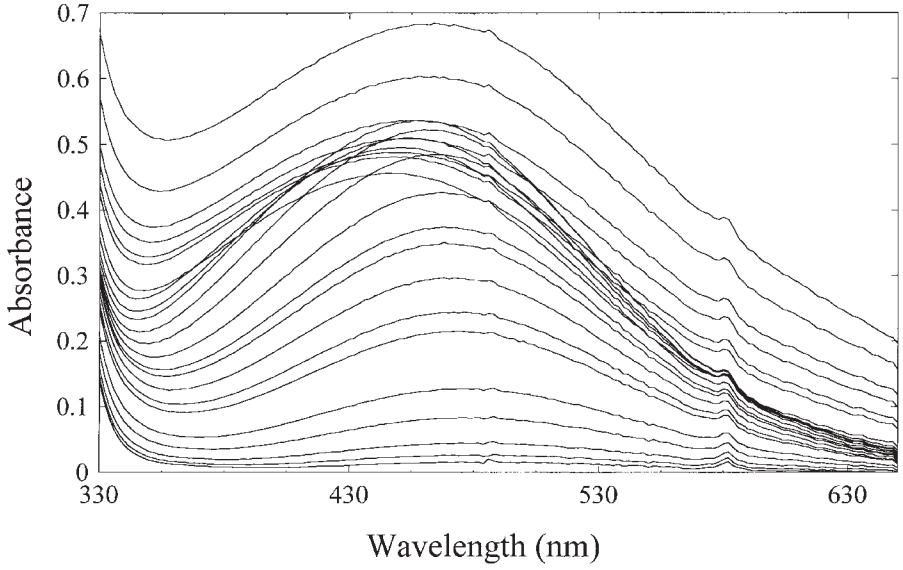


Figure 3. Solution spectra of the iron(III) complex with cystinedihydroxamic acid at various $[H^+]$. $[Fe^{3+}]_{tot} = 5 \times 10^{-3}$, $[CYS]_{tot} = 1 \times 10^{-3}$, $I = 0.1$ M (KNO_3) $T = 25$ °C, pH = 0.012–0.417.

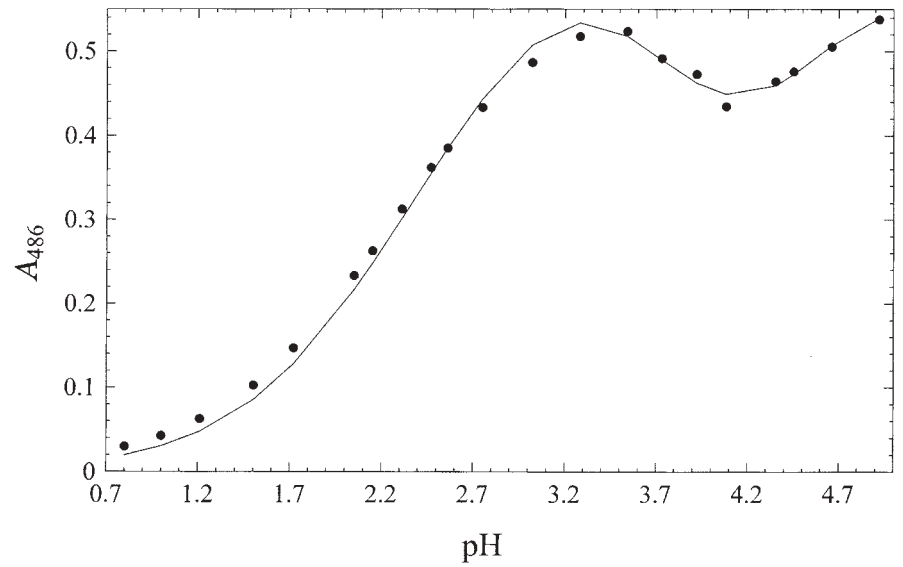


Figure 4. Dependence of the absorbance at 486 nm on pH of solution. Conditions as in Figure 3. The solid line represents fit of the data to the stability constants and molar absorptivities given in Table I.

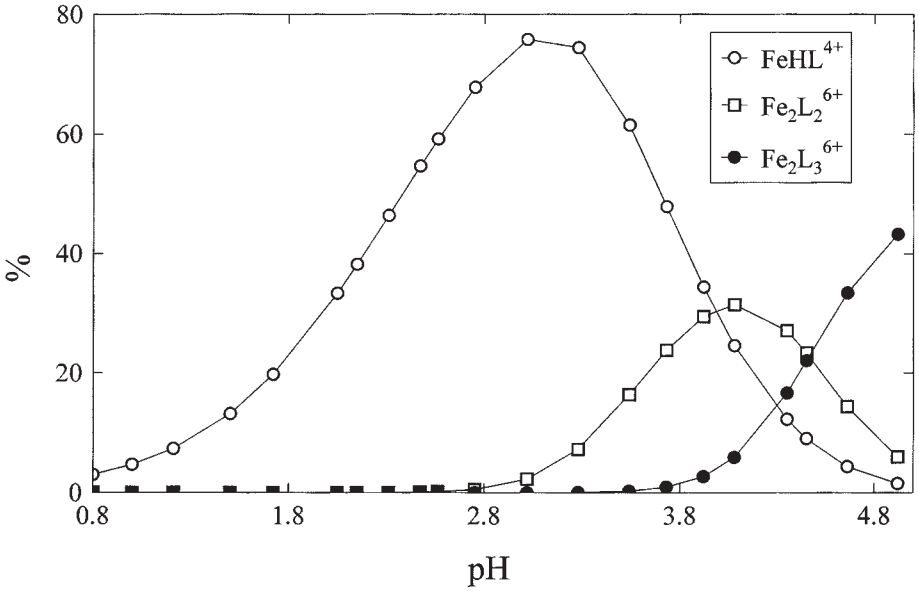


Figure 5. Distribution of different iron(III) species as a function of pH. Conditions as in Figure 3.

When the reaction solution pH was increased over 5, a dark purple complex precipitated. Analysis of this product showed Fe_2L_3 composition. An IR spectrum of this complex reveals absence of OH stretching frequency at 3038 cm^{-1} and a broad carbonyl stretching band at 1635 cm^{-1}, confirming a normal (O,O) coordination of the iron atoms with cystinedihydroxamic acid, like in the natural iron(III) complex with rhodotorulic acid. The carbonyl frequency in the complex is shifted by 26 cm^{-1} to a higher energy than that in the free ligand in solid state. The most likely explanation of this unexpected observation lies in the formation of strong intramolecular hydrogen bonding involving the carbonyl oxygen of one hydroxamic group with hydrogen atom of hydroxyl group of the other hydroxamic moiety.[23]

The X-ray structural reports on iron(III) complexes with hydroxamic acids show that complexation occurs by coordinating both the C=O and OH oxygen atoms of the hydroxamic group to the iron(III) centre forming the five-member chelate ring.[24] This happens with concomitant loss of the NOH proton. The presence of another functional group in a hydroxamic acid molecule, such as $-NH_2$ or S, offers the possibility of forming a complex in which such a group participates as electron pair donor.[25] However, the results presented above along with our earlier results with betainehydroxamic acid and glycinehydroxamic acid,[26] indicate that the coordination of iron through the $-NH_2$ or -S groups is not likely with cystinedihydroxamic acid.

EXPERIMENTAL

Cystinedihydroxamic acid was purchased from Sigma Chem. Co. (St.Louis) and its purity was checked potentiometrically. All other reagents were of analytical grade and were used without further purification. Double distilled water in an all-glass apparatus was used throughout the experiments.

Equilibrium absorbance data were collected using a Hewlett-Packard 8452A diode-array spectrophotometer. In spectrophotometric titrations, the solution was circulated to the spectrophotometer cell through polyethylene tubing with a peristaltic pump. Once equilibrium was established, the pump and stirrer were turned off just long enough to measure the pH and acquire spectra.

The IR spectra were obtained by using a FT-IR Perkin-Elmer Paragon 500 spectrometer from samples dispersed in KBr pellets.

The pH measurements were carried out in a water-jacketed cell maintained at 25 ± 0.1 °C by an external circulation bath. The Radiometer model PHM 85 digital pH-Meter with combination glass electrode (Radiometer GK 2322C) was used for pH readings.

The pK_a values of cystinedihydroxamic acid were determined by titrations with carbonate-free sodium hydroxide at ionic strength of 0.1 M (KNO_3) under a purified nitrogen atmosphere. All other experiments were performed in aqueous solutions of 0.1 or 1.0 M ionic strength (KNO_3) defined as 0.5 $\Sigma C_i \times Z_i^2$, where C_i and Z_i are total concentrations, and the formal charge numbers of the ions present in the solution, respectively.

The spectrophotometric data reduction analysis was performed using SPECFIT-program. A SUPERQUAD computer program was used for the refinement of pK_a values from the titration data.[27]

Preparation of Fe_2L_3

Cystinedihydroxamic acid (1.6391 g, 5.46 mmol) was dissolved in 300 mL water by addition of HNO_3 to pH 1.5. To this solution, $Fe(NO_3)_3 \times 9H_2O$ (735 mg, 1.82 mmol) dissolved in 50 mL of water was slowly added under constant stirring. Then, the pH of this solution was raised (pH 4) by dropwise addition of 1.0 M KOH. To this solution, saturated KNO_3 was added until precipitation occurred. The obtained product was allowed to stand overnight. The dark purple precipitate was filtered off, washed with water and dried in air and finely *in vacuo* over P_4O_{10} at room temperature for 72 h.

Anal. Calcd. for $C_{18}H_{36}N_{12}O_{12}S_6Fe_2 \cdot 7H2O$ M_r=(1042.7): Fe 10.71, S 18.45, C 20.73%; found: Fe 10.73, S 18.42, C 19.17%. IR(KBr): ν/cm^{-1} 3433 (NH)st, 1635 (CO)st.

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SAŽETAK

Kompleksacija željeza(III) sa cistindihidroksamnom kiselinom

Mladen Biruš, Suzana Inić, Nikola Kujundžić i Biljana Nigović

U kiselim i neutalnim otopinama cistindihidroksamna kiselina (H_2L^{2+}) veže željezov(III) ion stvarajući monomerne i dimerne komplekse u omjerima Fe : L = 1 : 1, 2 : 2 i 2 : 3. Usporedba ravnotežnih i spektralnih parametara mono(cistindihidroksamato) željezova(III) kompleksa s drugim željezovim(III) hidroksamatima upućuje na jednak način koordinacije. Izoliran je kompleks Fe_2L_3 i karakteriziran na osnovi elementne analize i IR-spektra.