obtained by reconstitution reactions of apoproteins with iron and selenium reagents. All such proteins exhibit effect i, but when they are compared to native proteins, the redox potential differences, while small, are variable in sign (−14 to +38 mV[33,34]). NMR and magnetic data have not been determined. At present firm evidence is lacking for the occurrence of selenium as a core constituent or as a terminal ligand (selenocysteinate) in Fe–S proteins of any type. However, the comparative data reported here for complexes **1**/**6** and **2**/**7**, as well as results for the protein 4-Fe site analogues $[Fe_4X_4(ZPh)_4]^{2-,3-}$ (X, Z = S, Se),[21] support the proposition that selenium could act as a functional substitute for sulfur in native proteins. Indeed, several Se-substituted 2-Fe proteins have proven active in bioassays.[30,32]

Lastly, it is observed that reaction 6 should be capable of extension to other aromatic thiols. The requirements are that the initially formed iron thiolate species be reactive toward elemental sulfur and that a suitable cation be employed to precipitate the desired 2-Fe complex before $[Fe_4S_4(SR)_4]^{2-}$ formation, by reaction 8 or other means, proceeds to an undesirable extent. A current limitation of the method is the inability to obtain $[Fe_2S_2(SR)_4]^{2-}$ complexes derived from monofunctional alkylthiols. Chelate complexes **1** and **6** are the only stable 2-Fe alkylthiolate species yet encountered. The first of these requirements also provides a limitation on $[Fe_4S_4(SR)_4]^{2-}$ synthesis by reaction 8, e.g., in the case of R = CH_2Ph at ambient temperature. It is the experience of this laboratory that virtually all iron alkyl- and arylthiolate species, including insoluble polymers, react with NaHS/NaOMe as the sulfide source in methanol at ambient temperature, in the absence of R'_4N^+ salts, to form $[Fe_4S_4(SR)_4]^{2-}$. The limiting stoichiometry is represented by reaction 9. In the R = alkyl

$$4FeCl_3 + 4NaHS + 6NaSR + 4NaOMe \rightarrow Na_2[Fe_4S_4(SR)_4] + RSSR + 12NaCl + 4MeOH \quad (9)$$

systems tested, co-introduction of the sulfide source and a R'_4N^+ salt, as in reaction 6, impeded further reaction to form tractable species (R = Et, *t*-Bu) or gave the tetrameric cluster (R = CH_2Ph) as the only identifiable product. A subsequent report will deal with the course of formation of $[Fe_4S_4(SR)_4]^{2-}$ by reaction 8, including the possible role of $[Fe_2S_2(SR)_4]^{2-}$ as an intermediate species.[18]

Acknowledgment. This research was supported by National Institutes of Health Grant GM22352. We thank Dr. G. Christou and K. S. Hagen for useful discussions and Dr. P. Mascharak for experimental assistance.

Registry No. $(Et_4N)_2[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]$, 56083-11-5; $(Et_4N)_2[Fe_2Se_2(S_2\text{-}o\text{-}xyl)_2]$, 74752-86-6; $(Et_4N)_2[Fe_2S_2(SPh)_4]$, 55939-70-3; $(Et_4N)_2[Fe_2Se_2(SPh)_4]$, 74752-88-8; $(Et_4N)_2[Fe_2S_2(SC_6H_4\text{-}p\text{-}Me)_4]$, 55939-64-5; $(Et_4N)_2[Fe_2Se_2(SC_6H_4\text{-}p\text{-}Me)_4]$, 74752-90-2.

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Design of Metal Chelates with Biological Activity. 2.[1] Solution Properties of Iron(III) Glycinehydroxamate

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Received March 4, 1980

Chemical criteria proposed previously as indicators of biological activity by a metal chelate as a source of a trace element are applied to ferric glycinehydroxamate, $Fe(GHA)_3$. The species distribution and the relevant stability constants of species present in aqueous solutions of $Fe(GHA)_3$ were obtained by analytical potentiometry. Together with magnetic susceptibility measurements, these results show the absence of any polymeric species at physiological pH values. The complex is shown also to donate iron rapidly to apotransferrin and the free ligand to effectively depolymerize iron citrate polymers. Biological potential is thus strongly indicated by all the proposed chemical criteria. In vitro studies indicate absorption of the undissociated chelate by rat intestines.

Introduction

This paper is the second part of a series devoted to finding in vitro chemical criteria which can be used as indicators of biological activity with the eventual aim of designing metal chelates as suitable sources of various trace elements essential in animal nutrition. In this paper, iron is the trace element considered, and we discuss ferric glycinehydroxamate as a possible source.

In living systems, the absorption, secretion, and retention of iron are largely controlled by the intestinal mucosa. A specific iron protein, transferrin, exists in the blood serum and serves as a carrier for iron.[2] Another iron protein, ferritin, functions both in iron absorption from the intestine and in iron storage in the liver, kidney, and spleen.[3] The normal iron content of men and women is 50 and 35 mg/kg of body weight respectively, giving an approximate total of 2–5 g of iron in the normal adult. When the level of iron falls below these levels, a state of iron deficiency is present. Apart from porphyrins, the other major class of naturally occurring iron complexing agents are the hydroxamic acids which complex as dihydroxamic acids (forming the mycobactins, mycelianamide, and pulcherrimic acid) and the trihydroxamic acids (forming the ferrichrome and ferrioxamine groups). These

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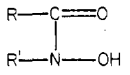
substances help to carry iron through metabolic channels and insert into porphyrins and other iron-containing enzymes and proteins.[4] In view of the importance of naturally occurring di- and trihydroxamic acids, our initial choice of possible biologically active iron chelates fell on complexes of monohydroxamic acids:



Both in vitro and in vivo studies of iron(III) acetohydroxamate (R = Me, R′ = H) have given promising results[1] with this complex showing a significant increase in regeneration of hemoglobin in anemic rats fed 2 mL of 4 mM iron(III) acetohydroxamate daily when compared to rats fed similarly with iron(III) citrate. The successful prediction of biological activity for a given iron chelate depends on an understanding of its function in biological systems and our ability to relate these functions to simple physicochemical properties of the chelates measured in vitro. This is a difficult task, but we have suggested that at least the following criteria should be met.[1] (1) The chelate must be stable and remain monomeric at biological pH values for transfer to occur through various cell membranes. (2) It must be able to undergo rapid iron exchange with apotransferrin since transferrin is responsible for iron transport in mammalian systems. (3) The free ligand should be able to extract iron fairly rapidly from ferritin, the iron-storage protein,[5] and as a model for this we have employed ferric citrate polymer.[7] Application of these chemical criteria to iron(III) glycinehydroxamate in an attempt to assess its potential as a suitable oral source of iron(III) is given below. In vivo studies will be reported separately.

Experimental Section

A. Materials. Glycinehydroxamic acid was obtained from Sigma & Co. (St. Louis), and its purity was checked potentiometrically. Distilled and deionized water was used throughout, and all titrations were carried out under an atmosphere of purified argon. The base used for pH measurements was carbonate-free sodium hydroxide (2.9927 M) and was standardized by using oven-dried potassium hydrogen phthalate. Stock solutions of $FeCl_3$ in dilute HCl were prepared from AnalaR $FeCl_3 \cdot 6H_2O$ and "Titrosol" HCl (Merck) stock solution. The stock Fe(III) solution was 0.099 19 M as determined by atomic absorption spectroscopy and was made up in 0.1 M HCl. All other reagents were of analytical grade (Ryvan Chemicals Ltd.).

Potentiometric titrations were performed by using a Radiometer (Copenhagen) automatic titration apparatus; pH meter readings were recorded on a Digital PHM64. Small amounts of base were added with the use of an ABU13 autoburette (volume 0.25 mL). Titrations were recorded graphically by using an TTT60 automatic titrator and automatic recorder REC61 servograph. The 50-mL solutions employed were thermostated to 25 ± 0.1 °C by using a water circulation pump. The electrode pair consisted of a Radiometer G2040C glass electrode and a K4050 KCl electrode. Absorption spectra in the region 800–300 nm were obtained on a Perkin-Elmer 124 spectrophotometer, and samples (2 mM) were scanned at a series of pH values from pH 2.0–10.0 at 25 °C by using 1-mm cells. Nuclear magnetic resonance spectra were obtained on a Perkin-Elmer R12B instrument. The method of Evans[6] was used to determine the magnetic moments of solutions in the pH range 2.0–11.0. Ferric citrate polymer was prepared as described earlier.[1] The depolymerization reaction was followed by monitoring the rate of appearance of the $Fe(GHA)_3$ complex (λ_{max} 425 nm). After an initial rapid increase in absorbance due to reaction with the 20% monomer known to be present in this ferric citrate polymer solution,[7] first-order kinetics were observed. Kinetics of the iron-transfer reaction between $Fe(GHA)_3$ and apotransferrin were followed at room temperature on a stopped-flow apparatus. Oscilloscope traces showed a biphasic reaction for changes in absorption at 470 nm with complex concentration of 2×10^{-4} M and protein concentration of 4×10^{-5} M before half-dilution on mixing. After dilution, there was a rapid decrease in transmitted light (0.1 s) followed by a slower first-order increase in transmittance due to the final product although there was some deviation from linearity toward the end so the rate constant quoted in Table III is an approximate value.

Table I. log Stability Constants ($\log \beta_{pqr}$) of Complex Species $M_pH_qA_r$ (M = Fe(III), A = Glycinehydroxamic Acid) in 0.15 M NaCl at 25 °C

			$\log \beta_{pqr}$	
			Sarkar-Kruck method	Irving-Rossotti method
p	q	r		
0	2	1	9.184	9.227
0	1	1	7.518	7.593
1	1	1	16.450	
1	0	1	13.388	13.410
1	1	2	27.558	
1	0	2	21.868	22.336
1	1	3	33.856	
1	0	3	26.501	25.537
1	−1	3	17.225	
1	−2	3	6.891	

In vitro absorption of the chelates by rat intestines was studied with use of 15 mL of Ringers solution as a model mammalian serum (960 mL of 0.154 M NaCl, 20 mL of 0.154 M KCl, and 20 mL of 0.11 M $CaCl_2$) at 38 °C with constant passage of 4.8% CO_2/O_2 mixture through the solution. The terminal ends of approximately 20-cm rat duodena from anemic Wister rats were tied with Ethilon polyamide blue monofilaments (45 cm) after removal from the rat abdomen. A total of 2 mL of 10 mM $Fe(GHA)_3$ chelate was injected into the isolated intestine. Contact with Ringers solution was maintained for 90 min, and the outer solutions were analyzed for chelate by measuring the absorption at λ_{max} 425 nm.

B. Methods. (1) Irving–Rossotti Method. The above standard technique[8,9] was used to determine both proton–ligand and metal–ligand formation constants. In the former case, titration curves were obtained by varying the initial concentration of glycinehydroxamic acid (C_A) from 0.50×10^{-2} to 2.50×10^{-2} M, in steps of 0.50×10^{-2} M in the presence of 0.10 M HCl (15 mL) and 1.50 M NaCl (5 mL). In the determination of metal–ligand constants, the concentration of HCl was kept constant (2.47×10^{-2} M) and two sets of curves were obtained. The first set was at constant ligand concentration ($C_A = 1.00 \times 10^{-2}$ M) with variation of metal concentration (C_M) from 2.50×10^{-4} M to 10.00×10^{-4} M in steps of 2.50×10^{-4} M. The second set was at constant metal concentration ($C_M = 5.00 \times 10^{-4}$ M) with variation of ligand concentration (C_A) from 5.00×10^{-3} M to 15.00×10^{-3} M in steps of 5.00×10^{-3} M. The resulting formation constants are given in Table I.

(2) Sarkar–Kruck Method. A method introduced by Österberg[10] for the measurement of free-ligand concentration during the formation of metal complexes has been extended by Sarkar and Kruck[11] to include the measurement of free-metal concentration. The calculations have been improved by the introduction of computer-based numerical procedures. Full details of the method are given in ref 11.

Proton–Glycinehydroxamic Acid System. A maximum number of two protons can be liberated from this ligand in the protonated form $N^+H_3CH_2CONHOH$ on titration with strong base in the pH range 1.5–11.0. If the pK_a values of the different buffering groups are separated by more than 2 log units, the Henderson–Hasselbach equation can be applied; however, in this case the buffering regions of the two groups (the OH of the NHOH and the NH_3^+) overlap so the above technique is inapplicable. The problem may be solved as follows. From the definition of $\bar{n}_H$[7,8] (see eq 1) rearrangement of

$$\bar{n}_H = \sum_0^Q q\beta q h^q / \sum_0^Q \beta q h^q \qquad (1)$$

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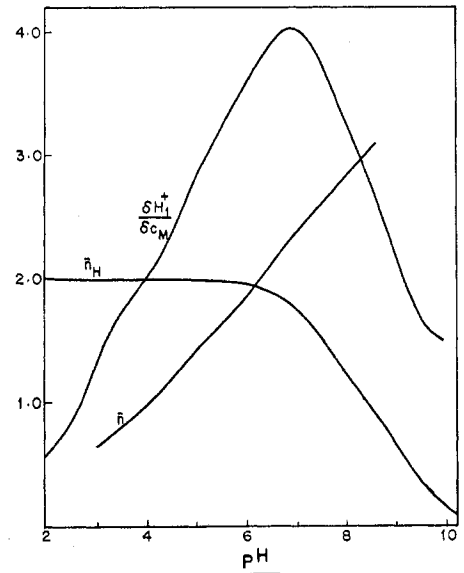

Figure 1. Molar proton liberation ($[H_1^+]/C_M$) and its relationship to $\bar{n}$ and $\bar{n}_H$.

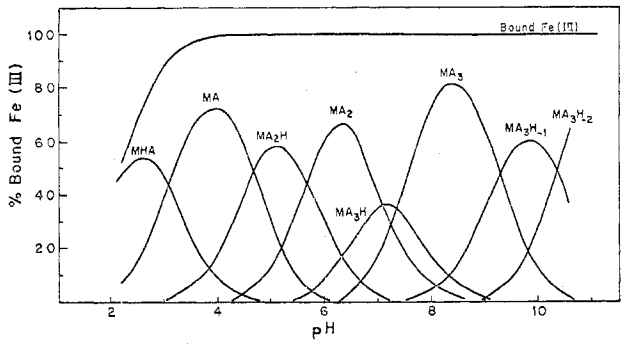

Figure 2. Species distribution in the binary system Fe(III)/GHA as a function of pH. $C_M = 5 \times 10^{-4}$ M; $C_A = 10^{-2}$ M.

(1) and taking the upper limit of the overlapping buffer region as $Q = 2$ gives eq 2 and hence eq 3 where $\bar{n}_H$ and h may be obtained from

$$\bar{n}_H = (\beta_1 h + 2\beta_2 h^2)/(1 + \beta_1 h + \beta_2 h^2) \quad (2)$$

$$\bar{n}_H = \beta_1(1 - \bar{n}_H)h + \beta_2(2 - \bar{n}_H)h^2 \quad (3)$$

a series of titration curves which differ only in their total ligand concentrations. Substituting $\bar{n}_H = Q - (\delta H_1^+/\delta C_A)$ into eq 2 gives eq 4 in the case $Q = 2$. The set of simultaneous equations (4) was

$$\left[2 - \frac{\delta[H_1^+]}{\delta C_A}\right] = \beta_1\left[\frac{\delta[H_1^+]}{\delta C_A} - 1\right]h + \beta_2\left[\frac{\delta[H_1^+]}{\delta C_A}\right]h^2 \quad (4)$$

solved by an iterative least-squares minimization method. Titration curves obtained on variation of ligand concentration were processed by the program PLOT II. The resulting proton liberation data, $\delta[H_1^+]/\delta C_A$ vs. pH, was substituted in eq 4 which was solved for β_1 and β_2 as above. The refined values so obtained are listed in Table I.

The titration curves obtained on variation of metal concentration were processed by PLOT II to give $\delta[H_1^+]/\delta C_M$ as a function of pH (Figure 1). Finally, the program GUESS II was applied to the results of $P_A = f(pH)$ and $P_M = f(pH)$ computed above by PLOT II to obtain the species distribution $M_pH_qA_r$, the following trial values of p, q, and r being selected in this case: $p = 1$, $q = 0$–5, $r = 1$–3. The refined values of the stability constants for the species thus obtained by minimum error solution of the matrix A_{ij} [with elements $a_{ij} = (m^p h^q a^r)_{ij}$] are given in Table I. The species distribution for the initial concentrations $C_M = 5 \times 10^{-4}$ M and $C_A = 10^{-2}$ M is given in Figure 2.

Electronic Spectra/pH Profile. The spectra of mixed metal/ligand solutions ($C_M = 2 \times 10^{-3}$ M, $C_A = 2 \times 10^{-2}$ M) with the pH adjusted to the same values in the range 2.5–11.0 as those used in the species distribution studies were measured in the visible region (400–800 nm). At low pH (2.5), the solution showed λ_{max} at 470 nm, but with increase in pH, there was a gradual increase in absorption and shift at λ_{max} to 425 nm. At pH > 11.0 the solutions showed a decrease in absorption, and precipitation occurred on standing.

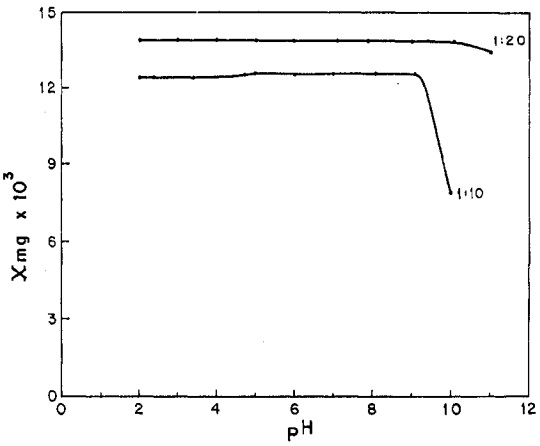

Figure 3. Dependence of $\chi_g \times 10^3$ (molar susceptibility) of Fe(III) in the Fe(III)/GHA system as a function of pH. 1:10 ratio: $C_M = 2.00 \times 10^{-3}$ M; $C_A = 2.00 \times 10^{-2}$ M. 1:20 ratio: $C_M = 2.00 \times 10^{-3}$ M; $C_A = 4.00 \times 10^{-2}$ M.

Results and Discussion

State of Aggregation. The magnetic moments of solutions of iron(III) glycinehydroxamic acid are given as a function of pH for various [ligand]/[metal] ratios (Figure 3). For a [ligand]/[metal] ratio of 10:1 and for pH >9.0, there is a marked reduction in μ_{eff} calculated on the basis of a monomeric Fe(III) complex. In contrast, for [ligand]/[metal] ratios greater than 10:1 no reduction in moment occurs until highly alkaline pH values (>12.0). On the assumption that a reduction in magnetic moment implies metal–metal interaction as in a polymeric species, it follows that for [ligand]/[metal] ratios >10:1 the chelate is present as a monomer.

Species Distribution. The species distribution for a metal concentration of $C_M = 5 \times 10^{-4}$ M and ligand concentration of $C_A = 10^{-2}$ M is given in Figure 2, and the stability constants of the various species are given in Table I. In the normal aqueous titration range, glycinehydroxamic acid can liberate two protons, one from the protonated amino group (NH_3^+), pK_1^H, and one from the OH group of the hydroxamic group (NHOH), pK_2^H. These show buffering behavior in the pH range 7.5–9.5. The final refined values of pK_1^H and pK_2^H are 9.18 and 7.52, respectively. The presence of the α-amino group in the aminohydroxamic acid increases the acid character of the OH group in comparison with that of acetohydroxamic acid ($pK^H = 9.342$[1]). Similarly, comparison of our pK_1^H for glycinehydroxamic acid with that of glycine $pK_1^H = 9.76$[12] shows that substitution of an NHOH group for the OH group of a carboxyl lowers the pK_a substantially in accord with the electron-withdrawing character of the NHOH group. Previously reported pK_1^H and pK_2^H values for GHA were 9.1 and 7.10, respectively, due to Cielesky and co-workers.[13]

In the presence of Fe(III), the proton liberation data (Figure 1) shows that complexation with Fe(III) begins at low pH values (<2.0), indicating that complexation at pH 2.4 presumably gives an MHA species. The proton displacement value reaches a maximum of 4.0 protons/mol of Fe(III) at pH 6.8 when the species MA_2 is predominant (Figure 2) which is equivalent to *two* protons being liberated on coordination

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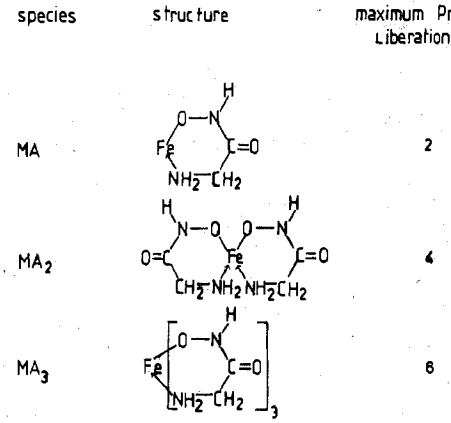

Figure 4. Proposed structures of various species in the Fe(III)/GHA system.

of *one* ligand molecule. This result can be explained by assuming that glycinehydroxamic acid acts as a bidentate ligand coordinating to Fe(III) via the OH group of NHOH and the α-amino group in contrast to the normal coordination of unsubstituted monohydroxamic acids.[14] The proposed structures of the major species based on the above assumption are given in Figure 4. The distribution shows various species MHA, MA, MHA_2, and MA_2 occurring in the pH region 2.0–6.8. With a pH beyond 6, the proton liberation curve starts to fall. For the species MA_3 at pH 8.0, approximately 3.5 protons/mol of Fe(III) are liberated, and at this pH the NHOH hydroxyl has lost 90% of its protons. Above pH 9.0, two hydroxyl species were detected. In the various protonated species, e.g., MHA and MHA_2, protonation may occur either at the amino group or at the hydroxamate ($N-O^-$) group. If the coordination pattern observed in the major species is presumed to hold for the protonated species, then the latter type of protonation occurs, but it is not possible to assign unambiguously the structures of these species from the solution data although the electronic spectra give some support for this view (see below). However, whatever the structure of the protonated species, it is clear that the major species (MA_2 and MA_3) in the physiological pH range (6.5–7.5) do not show normal hydroxamate coordination[14] but instead display coordination by the α-amino group and the OH group of NHOH. The species distribution shows also that in the above pH range the bis chelate MA_2 accounts for over 75% of the species present, and there is no evidence for the presence of *any* polymeric species.

Finally, it is gratifying that the stability constants calculated by both methods agree closely (Table I), but it should be stressed that the method of Sarkar and Kruck possesses the great advantage of giving an accurate species distribution as a function of pH.

Electronic Spectra/pH Profile. The spectra are shown for Figure 5 for the pH range 2.5–9.0. The highest limiting λ_{max} for the Fe^{3+}/GHA system is approximately 485 nm whereas for the Fe^{3+}/AHA system λ_{max} reaches 500 nm, suggesting that even in the protonated species the mode of coordination of GHA is different from that of AHA. By comparison with the structures of the major species (MA_2, MA_3), we suggest that coordination in MHA and MHA_2 also involves the amino group and the hydroxyl of the NHOH group. The increase in absorption and shift to smaller wavelengths with increase in pH indicates greater complexation, and, above pH 7.0, both λ_{max} and ϵ_{max} remain constant, consistent with formation of the MA_3 species which predominates in this range. At pH >9.0, there is a marked decrease in absorption when the MA_3H_{-1} and MA_3H_{-2} species form.

Table II. Depolymerization Kinetics for FeCit(polymeric) + L → FeL + Cit

ligand (L)	[FeCit]/[L]	$10^5 k_{obsd}$, s^{-1}	ref
NaH_2Cit	1:50	4.775	7
Na_2H_2(EDTA)	1:50	3.166	7
Na_2H Cit	1:50	1.972	7
acetohydroxamic acid (AHA)	1:100	35.59	1
glycinehydroxamic acid (GHA)	1:100	22.68	

Table III. Rates of Fe^{3+} Exchange between Chelates and Apotransferrin

chelate	k_{obsd}	ref
Fe(EDTA)	0.85×10^{-3} min^{-1}	15
FeCit	2.5×10^{-3} min^{-1}	15
Fe(NTA)	5.5×10^{-1} s^{-1}	17
Fe(AHA)	1.59×10^{-2} s^{-1}	1
Fe(GHA)	1.7×10^{-1} s^{-1}	

Table IV. In Vitro Intestinal Absorptions in Rats as Percentage of Dose[a]

	Fe chelates	
	AHA	GHA
% abs	21 ± 5	21 ± 5
metal:ligand ratio	1:5	1:20

[a] Concentration = 10 mM; pH 7.3.

Depolymerization Kinetics. The kinetic data for the depolymerization of the ferric citrate polymer are given in Table II. An initial rapid increase in absorbancy is followed by a first-order increase from which the rate constants were derived. The fraction of total absorbance charge due to the initial rapid increase was ~20% (estimated by extrapolating the first-order curve to zero time) which is in good agreement with the 20% monomer reported previously in the polymer solution.[7] For glycinehydroxamic acid the rate constants are about 10 times those of other well-known chelates for Fe(III) and are similar in magnitude to that of acetohydroxamic acid.[1] At pH 8.5, the percentage of MA_3 species is about 60% for acetohydroxamic acid[1] and 82% for GHA; however, the higher basicity of the two groups in GHA makes it a smaller depolymerizing agent to AHA and so both ligands show similar specificity for iron, a conclusion which is supported by the similar values of log β_{103} for $Fe(GHA)_3$ (26.5) and for $Fe(AHA)_3$ (24.62).

Kinetics of Iron Transfer to Apotransferrin. The rate at which iron is donated to apotransferrin by an iron chelate is highly dependent on the nature of the chelate but unrelated to its stability constant.[15] Rapid rate measurements again showed a biphasic reaction as reported previously for $Fe(AHA)_3$.[1] The simplest reaction sequence which accounts for this is

$$Fe(GHA) + \text{apotransferrin} \rightleftharpoons \text{intermediate} \rightleftharpoons \text{iron transferrin} + GHA$$

In the initial phase (~100 ms) the chelate reacts with apotransferrin probably to form a ternary complex[17] which then decays in the second phase (~20 s) to form iron transferrin and free ligand. The order of reactivites for a series of Fe(III) chelates is given in Table III. Clearly, Fe(GHA) donates its iron very rapidly and even faster than Fe(AHA) which has

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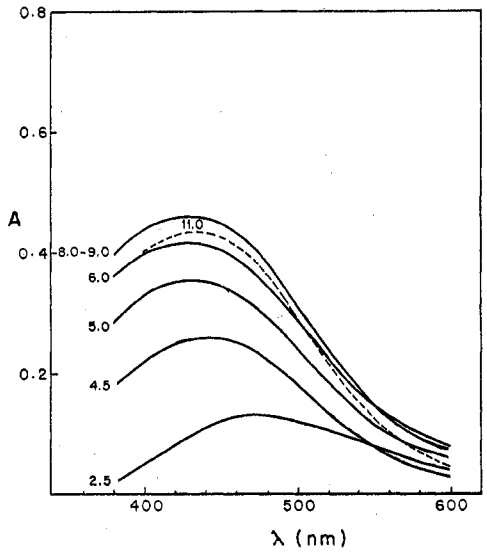

Figure 5. Visible spectra of Fe(III)/GHA as a function of pH. $C_M = 2.00 \times 10^{-3}$ M; $C_A = 2.00 \times 10^{-2}$ M (1-mm cells).

already been shown to possess a high ability to regenerate hemoglobin levels in anemic rats.[1]

In Vitro Absorption. Table IV shows interestingly that 15–20% of the Fe(GHA) chelate is absorbed (in 90 min) by rat intestines, and this result is similar to that of Fe(AHA). Moreover, the appearance of *undissociated* metal chelate in the Ringers solution suggests that the chelate remains stable in the intestine after initial absorption and on migration through cell membrane of the intestine.

Conclusion. The above potentiometric, spectroscopic, and magnetic results show that glycinehydroxamic acid coordinates to Fe(III) via the α-amino nitrogen and the hydroxyl oxygen of the NHOH group and that aqueous solutions contain monomeric species in the pH range 2.0–10.0. The kinetic criteria show effective depolymerization of the ferric citrate polymer by GHA and very rapid donation of iron to apotransferrin by the chelate. We conclude therefore that the Fe(III)/GHA system satisfies all the criteria for biological activity proposed,[1] giving a strong indication for Fe(GHA) as a suitable source of iron as a trace element. Animal studies are presently in progress to test this prediction based on chemical criteria. Finally, it has been suggested that aminohydroxamic acids may be useful in the treatment of iron-overload diseases[16] with emphasis on the surface-active role of an uncoordinated amino group. Our results show clearly that, at least in the Fe/GHA system at physiological pH values, the assumption of an uncoordinated α-amino group is incorrect since the major species in this pH region is MA_2 which involves coordination of the α-amino group and the hydroxamate OH group.

Acknowledgment. M.V.C. thanks the Department of Education for the award of a Postdoctoral Fellowship during the tenure of which the work was carried out. We should also like to thank Professor D. McAleese and Dr. J. J. Clarke of the Department of Agricultural Chemistry, University College, Dublin, for their kind assistance with in vitro experiments.

Registry No. $Fe(GHA)_3$, 74610-16-5; GHA, 5349-80-4; AHA, 546-88-3; FeCit, 28633-45-6.

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A Tensimetric Titration Study of Adduct Formation Involving the Lewis Acids $Cl_xGa(n\text{-}C_4H_9)_{3-x}$ and the Lewis Bases $(CH_3)_zNH_{3-z}$ Where x and z = 0–3[1]

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Received August 30, 1979

The Lewis acid–base reactions of a series of butylgallium chlorides, $Cl_xGa(n\text{-}C_4H_9)_{3-x}$, and methylamines, $(CH_3)_zNH_{3-z}$, where x and z = 0–3, were studied in toluene by using a tensimetric technique. Adduct formation was found to be dependent on x, z, and the reaction temperature, with acid affinity toward base decreasing in the order $GaCl_3 > Cl_2GaC_4H_9 > ClGa(C_4H_9)_2 > Ga(C_4H_9)_3$ and with base affinity toward acid decreasing in the order $NH_3 > CH_3NH_2 > (CH_3)_2NH > (CH_3)_3N$. These results are explained by steric and/or inductive effects in the acids and steric, back-strain effects in the bases. The general structural model $[Cl_{x-y+1}GaR_{3-x}B_y]^{y-1},(y-1)Cl^-$, where y is the base/acid mole ratio in the adduct and B is base, is proposed for all adducts.

The *Lewis acid properties* of a main-group organometallic are fundamental to its entire chemistry. Although the literature contains several references to selected gallium compounds[4] and comparison with organometallics of other main-group metals, no systematic study of a homologous series of organogallium compounds has appeared. Therefore, the relative importance of such factors as "*front* strain", "*back*

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