The Design of Metal Chelates with Biological Activity. Part 6*. Nickel and Iron Complexes of Glycylglycinehydroxamic Acid and Triglycinehydroxamic Acid

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Abstract

Monohydroxamic acids of the peptides glycylglycine and triglycylglycine are reported and fully characterised. Their NMR spectra indicate the presence of *Z* and *E* isomers analogous to those recently reported for the simple monoalkylhydroxamic acids. Species distribution analyses and stability constant data for complex species present in aqueous solution for the interaction of these peptide hydroxamic acids with Ni(II) and Fe(III) were obtained by analytical potentiometry. Spectroscopic data confirm normal hydroxamate coordination via the ketonic carbonyl oxygen atom and the deprotonated hydroxamate oxygen atom in these metal complexes.

Introduction

This paper is part of a series devoted to the synthesis of metal chelates with biological activity, in particular a series of iron complexes which may be capable of oral administration in the treatment of anemia. Since naturally occurring hydroxamic acids (siderophores) are involved in microbical transport of iron [2] we have concentrated our attention on complexes of various types of hydroxamic acids, including alkyl [3], amino [4] and dihydroxamic acids [1]. In addition to being intimately involved in the transport and storage of iron in mammalian systems, hydroxamic acids are also potent and specific inhibitors of urease activity [5] and have been used therapeutically in the treatment of hepatic coma [6]. The inhibition of urease by hydroxamic acids has been interpreted in terms of reversible binding to the active site nickel ion [7], consequently, it is important to investigate the complexing ability of hydroxamic acids not only with iron but also with nickel. In the present paper, we report a detailed solution study of the interaction of iron(III) and nickel(II) with the peptide monohydroxamic acids, glycylglycine hydroxamic acid ($n = 1$) and triglycinehydroxamic acid ($n = 2$), $H_2NCH_2(CONHCH_2)_nCONHOH$ (**A**) together with the isolation of the iron (1:3) complexes FeA_3 and the nickel (1:2) complexes NiA_2 in the solid state.

Experimental

Melting points were determined using a Gallenkamp melting point apparatus. ^1H NMR spectra were recorded at 270 MHz using a Jeol GX270 instrument with tetramethylsilane as an internal standard. The ^{13}C NMR spectra were recorded at 67.80 MHz using the same instrument. The IR spectra were recorded on a Perkin-Elmer 1710 Infrared Fourier Transform spectrometer (as 2% KBr disks) and UV–Vis spectra on a Perkin-Elmer 552 spectrophotometer. Magnetic moments were measured on a Newport variable-temperature Gouy balance calibrated with cobalt(II) tetrakis(thiocyanato) mercurate(II).

Synthesis of Hydroxamic Acids

Glycylglycine hydroxamic acid and triglycine hydroxamic acid were synthesised by dropwise addition of the respective ethyl esters (prepared by addition of equimolar alcoholic KOH to a suspension of ester hydrochloride in ethanol) to hydroxylamine in ethanol.

Glycylglycine hydroxamic acid

The synthesis of glycylglycine hydroxamic acid was difficult due to the formation of glycine anhydride (2,5-piperazindione) as a side product at pH $\geq$ 12. However, careful control of temperature (5–10 °C) and pH (pH $\leq$ 11) gave glycylglycine hydroxamic acid in 49% yield, melting point (m.p.)/decomposition point 175–176 °C. *Anal.* Found: C,

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32.20; H, 6.20; N, 28.19. Calc.: C, 32.65; H, 6.12; N, 28.55%. ^{1}H NMR (DMSO-d_6): major peaks, δ8.05 (S, 1H, NH), δ3.65 (S, 1.85H, NH_2–C), δ3.42 (bS, NH_2) and δ3.11 (S, 1.85H, NH–CH_2–C–) a minor peak, δ3.96 (S, 0.15H, CH_2). ^{13}C NMR (DMSO-d_6): major peaks, 171.3 (*C*), 44.2 (*C*H_2), 41.5 (*C*H_2) ppm and minor peaks, 171.4 (*C*), 167.7 (*C*), 43.7 (*C*H_2) and 41.3 (*C*H_2) ppm. IR spectrum (solid 2% KBr disk): 3440 (bsh, OH), 3280 (S, NH), 3212 (sh, NH), 1673 (VS, carbonyl), 1646 (VS, carbonyl) cm^{-1}.

Triglycine ethyl ester hydrochloride

Triglycine ethyl ester hydrochloride was synthesised by passing dry HCl into a suspension of triglycine in ethanol. The resulting mixture was stirred overnight at room temperature, evaporated to dryness and recrystallised from ethanol to give triglycine ethylester hydrochloride in 96% yield, m.p. 205 °C. *Anal.* Found: C, 37.97; H, 6.09; N, 16.29; Cl, 14.15. Calc.: C, 37.87; H, 6.31; N, 16.57; Cl, 14.00%. ^{1}H NMR (D_2O); δ4.22 (q, J = 7 Hz, 2H, CO_2CH_2Me), δ4.06 (S, 2H, NH_3CH_2), δ4.03 (S, 2H, $HNCH_2CO$), δ3.90 (S, 2H, CH_2CO_2Et) and δ1.26 (t, J = 7 Hz, 3H, CH_3). ^{13}C NMR (D_2O): 174.4 (*C*), 174.3 (*C*), 170.5 (CO_2Et), 65.3 (OCH_2), 44.9 (*C*H_2), 44.0 (*C*H_2), 43.1 (*C*H_2) and 16.0 (CH_3) ppm. IR spectrum (solid, 2% KBr disk): 3329 (m, NH), 3227 (m, NH), 1735 (VS, ester carbonyl), 1646 (VS, amide carbonyl) cm^{-1}.

Triglycine hydroxamic acid

The dropwise addition of triglycine ethyl ester (prepared by addition of equimolar amounts of alcoholic KOH to a suspension of ester hydrochloride in ethanol) to hydroxylamine in ethanol gave triglycine hydroxamic acid, in 78% yield, decomp. point 185 °C. *Anal.* Found: C, 35.37; H, 5.84; N, 27.56. Calc.: C, 35.29; H, 5.88; N, 27.45%. ^{1}H NMR (DMSO-d_6): major peaks, δ8.16 (bS, 2H, N*H*), δ3.75 (S, 1.85H, H_2NCH_2), δ3.62 (d, 1.85H, J = 5.5 Hz, CH_2), δ3.35 (bS, NH_2) and δ3.13 (S, 1.85H, CH_2); minor peaks, δ3.96 (S, 0.15H, CH_2), δ3.83 (d, J = 5.9 Hz, 0.15H, CH_2). ^{13}C NMR (DMSO-d_6): major peaks 173.3 (*C*), 169.2 (*C*), 165.4 (*C*), 44.6 (*C*H_2), 41.7 (*C*H_2) ppm; minor peaks, 173.4 (*C*), 170.7 (*C*), 167.4 (*C*), 43.7 (*C*H_2) and 41.4 (*C*H_2) ppm. IR spectrum (solid, 2% KBr disk): 3450 (bS, OH), 3279 (S, NH), 3212 (sh, NH), 1672 (VS, carbonyl), 1646 (VS, carbonyl) cm^{-1}. The NH_2 and NH peaks in ^{1}H NMR (DMSO-d_6) disappeared on addition of six drops of D_2O. The D_2O shake showed another minor peak at δ3.45 (which was masked previously by the NH_2 peak); the major peaks at δ3.75, δ3.62 and δ3.13 were shifted to δ3.80, δ3.67 and δ3.18 respectively.

Preparation of Ni(II)/Hydroxamic Acid Complexes

(i) Preparation of Ni(glyglyHA)$_2$·H_2O

An aqueous solution of glycylglycine hydroxamic acid (0.600 g, 0.042 mol) was added to an aqueous solution of $NiCl_2$·$6H_2O$ (0.476 g, 0.02 mol). The pH of the solution was raised to 7.60 using aqueous potassium hydroxide. The resulting solution was stirred at 0–5 °C for two hours and ethanol was added to give a green solid (0.564 mg, 76% yield). *Anal.* Found: C, 25.92; H, 4.98; N, 22.68; Ni, 15.86. Calc.: for Ni (glygly HA)$_2$·H_2O: C, 26.02; H, 4.88; N, 22.76; Ni, 15.91%. IR (solid, 2% KBr disk): 3416 (sh, OH of water); 3286 (S, NH); 3100 (sh); 1644 (S, carbonyl); 1582 (S, carbonyl bonded to Ni) cm^{-1}. Magnetic moment at 20 °C is 2.94 BM. UV–Vis spectrum (solid): 625, 476 (sh) and 402 nm; UV–Vis (aq. solu.): 625 (Vb); 478 (sh) and 402 nm.

(ii) Preparation of Ni(trigly HA)$_2$·H_2O

An aqueous solution of triglycine hydroxamic acid (0.850 g, 0.042 mol) was added to an aqueous solution of $NiCl_2$·$6H_2O$ (0.476 g, 0.02 mol). The pH of the solution was raised to 7.80 using aqueous potassium hydroxide. Addition of ethanol after stirring the resulting solution at 0–5 °C for 2 h, gave a green solid (0.668 g, 69% yield). *Anal.* Found: C, 30.06; H, 5.22; N, 22.98; Ni, 11.91. Calc. for Ni-(trigly HA)$_2$·H_2O: C, 29.83; H, 4.97; N, 23.20; Ni, 12.16%. IR (solid, 2% KBr disk): 3420 (sh, OH of water); 3289 (S, NH); 3091 (sh); 1647 (S, carbonyl); 1568 (S, carbonyl bonded to Ni) cm^{-1}. Magnetic moment at 20 °C = 2.93 BM. UV–Vis spectrum (solid): 620, 478 (sh) and 403 nm; (aq. sol.): 620 (Vb), 480 (sh) and 403 nm.

Preparation of Fe(III)/Hydroxamic Acid Complexes

(i) Preparation of Fe(glyglyHA)$_3$

An aqueous solution of glycylglycine hydroxamic acid (0.900 g, 0.0063 mol) was added to an aqueous solution of $FeCl_3$·$6H_2O$ (0.540 g, 0.002 mol). The pH of the solution was raised to 8.00 using aqueous potassium hydroxide. The resulting solution was stirred at 0–5 °C for 2 h and ethanol was added to give a red solid (0.788 mg, 80% yield). *Anal.* Found: C, 29.11; H, 4.88; N, 25.26; Fe, 11.38. Calc.: C, 29.15; H, 4.86; N, 25.51; Fe, 11.34%. IR (2% KBr disk): 3286 (sNH); 1665 (s, carbonyl); 1571 (s, carbonyl bonded to Fe) cm^{-1}; magnetic moment at 20 °C = 5.27 BM. Visible spectrum (solid and aq. solu.): 426 and 388 nm.

(ii) Preparation of Fe(triglyHA)$_3$

An aqueous solution of triglycine hydroxamic acid (1.240 g, 0.0063 mol) was added to an aqueous solution of $FeCl_3$·$6H_2O$ (0.540 g, 0.002 mol). The pH of the solution was raised to 8.00 using aqueous potassium hydroxide. The resulting solution was stirred at 0–5 °C for 2 h and ethanol was added to give a red solid (0.868 g, 66% yield). *Anal.* Found: C, 32.46; H, 4.84; N, 25.15; Fe, 8.56. Calc.: C, 32.63; H, 4.98; N, 25.38; Fe, 8.46%. IR (2% KBr disk):

3280 (s, NH); 1661 (s, carbonyl); 1549 (s, carbonyl bonded to Fe) cm^{-1}; magnetic moment at 20 °C = 5.281 BM. UV–Vis spectrum (solid, aq. solu.): 427 and 390 nm.

Potentiometric Titrations

Distilled deionised water was used and all titrations carried out under an atmosphere of purified argon. The base for pH measurement was carbonate free sodium hydroxide (0.25 M) prepared from standard convol (BDH) sodium hydroxide solution and was standardised with oven-dried potassium hydrogen phthalate. Stock solutions of ferric chloride were prepared from 'Titrosol' $FeCl_3$ (Merck) solution. A stock solution of $NiCl_2$ (6×10^{-3} M, as determined by atomic absorption spectroscopy) was prepared from Analar $NiCl_2 \cdot 6H_2O$. Standard convol (BDH) HCl stock solution was used and all other reagents were of analytical grade.

Potentiometric titrations were performed using a Mettler DL20 compact Titrator connected to an Epson LX80 printer where the pH readings and volume of base added were recorded automatically. Small amounts (0.01 ml) of base were added with the use of a DV410 autoburette. The 50 ml solutions employed in all titrations were thermostatted to 25 ± 0.1 °C using a water circulation pump. A Mettler DG111 combination glass electrode was used and calibrated with buffer solutions pH 4 and pH at 25 °C. Titrations were carried out with a 0.2 M KCl solution as a background electrolyte to keep the ionic strength constant.

Method. The Sarkar–Kruck method, a method introduced by Osterberg [8] for the measurement of free-ligand concentration during the formation of metal complexes and extended by Sarkar and Kruck [9] to include the measurement of free-metal concentration was used. The calculations have been improved by the introduction of computer-based numerical procedures. Full details of the method are given in ref. 9. Sample compositions of the various ligands and metal ions used are given in the Tables, pK values are accurate to ±0.02 units.

Results and Discussion

Hydroxamic acids, are known to exist (in solution) as both Z and E isomers [10] with the Z isomer predominating in DMSO-d_6 solution. For example, acetohydroxamic acid $CH_3CONHOH$ shows two sets of peaks at room temperature with an intensity ratio of approximately 9:1. The major peak at δ10.36 was confirmed as due to the NH proton by ^{15}N labelling experiments and that at δ8.69 to the OH peak of the Z isomer whilst the respective minor peaks at δ9.80 and δ9.13 were assigned to the NH and OH protons of the E isomer. The presence of two similar sets of peaks (see 'Experimental') in the ^1H and ^{13}C NMR spectra of $H_2NCH_2(CONH_2)_nCONHOH$ ($n = 1, 2$) suggests analogously the presence of Z and E isomers in DMSO-d_6 for these peptide hydroxamic acids also.

pK_a Calculation

The pK_a values of the protonated amino groups and the hydroxamate hydroxyl groups for glycylglycine hydroxamic acid, $H_2NCH_2CONHCH_2CONHOH$ and triglycine hydroxamic acid, $H_2NCH_2(CONHCH_2)_2CONHOH$ in aqueous solutions were measured by potentiometric titration and subsequent analysis using the iterative least-squares programme LEASK II [9]. The titration details are given in Table 1 (see also 'Supplementary Material'). The pK_a values obtained by this method are 7.52 and 8.40 for glycylglycine hydroxamic acid and 7.51 and 8.40 for triglycine hydroxamic acid (see Table 3). (The pK_a values of 7.52 and 9.18 for glycine hydroxamic acid [4] are also given in Table 3 for comparison).

Ni(II) Complexes

Both glycylglycine hydroxamic acid and triglycine hydroxamic acid form green 1:2 complexes, $Ni(H_2NCH_2(CONHCH_2)_nCONHO)_2 \cdot H_2O$ ($n = 1, 2$) with nickel(II) at pH 7.80. Their infrared spectra show shifts of 80–100 cm^{-1} for the ketonic carbonyl frequency compared to those of the free ligands which argues strongly for complexation through the ketonic oxygen atom. Similarly, the electronic spectrum of $Ni(GlyGlyHA)_2H_2O$ exhibits bands with max. at 402 and 625 nm with a shoulder at 478 (solid state) or 480 (aq. solu.) nm and that of $Ni(GlyGlyGlyHA)_2H_2O$ similar max. at 403 and 620 nm and a shoulder at 478 (solid) or 480 (aq. solu.) nm which compare closely with those of $Ni(AHA)_2 \cdot 2H_2O$ (AHA = acetohydroxamic acid) at 389 and 667 nm whereas $Ni(GlyHA)_2$ exhibits max. at 450 nm [3]. The above spectroscopic evidence shows clearly that coordination in the nickel peptide hydroxamic acids involves normal hydroxamate bonding via the carbonyl oxygen and deprotonated NHO^- group as reported [11] for a range of transition metal complexes of alkyl hydroxamic acids. The solid state

TABLE 1. Concentration variation (mol l^{-1}) during the pK_a determination of $H_2NCH_2(CONHCH_2)_nCONHOH$

$C_{H_2NCH_2(CONHCH_2)_nCONHOH}$	
$n = 1$	$n = 2$
3.06×10^{-3}	3.21×10^{-3}
6.12×10^{-3}	6.42×10^{-3}
9.18×10^{-3}	9.63×10^{-3}

$C_{NaOH} = 0.25$ M; $C_{HCl} = 0.02$ M; $C_{KCl} = 0.2$ M. Temperature = 25 ± 0.1 °C; argon atmosphere; total volume = 50 ml.

magnetic moments of $Ni(GlyGlyHA)_2 \cdot H_2O$ (μ = 2.94 BM at 20 °C) and of $Ni(GlyGlyGlyHA)_2 \cdot H_2O$ (μ = 2.93 BM, 20 °C) and their relative insensitivity to decrease in temperature (down to −180 °C) suggests octahedral coordination similar to that of the bis alkylhydroxamates of nickel(II) [3] although in the present case of the nickel peptidehydroxamates, the sixth coordination position may be occupied by the nitrogen atom of the amino group, a suggestion which is supported by the observed shoulders at 480 nm in the electronic spectra which may indicate a slightly distorted octahedral structure.

Species Distribution in the Ni(II)/Hydroxamic Acid System

In aqueous solution, the nature of the species present will depend upon the pH. The species distribution and the stability constants of the complex species in the $Ni(II)/H_2NHC_2(CONHCH_2)_nCONHOH$ (n = 1, 2) systems were analysed in the pH range 4–11 by the method of Sarkar and Kruck [9] from potentiometric titration data details of which are given in Table 2 (see also 'Supplementary Material'). The species distributions are given as a function of pH in Figs. 1 and 2 respectively together with the stability constants of the various species in Table 3. In Fig. 1, it can be seen that the following species were detected for the Ni(II)/glycylglycine hydroxamic acid system: M_2A, MA_2H_2, MA, MA_3H_3, MA_2, MA_2H_{-1} and MA_2H_{-2}. In the acidic region, two protonated complexes MA_2H_2 and MA_3H_3 and two deprotonated complexes M_2A and MA are formed; the protonated complex MA_3H_3 and the deprotonated 1:1 complex MA are the major complex species in equilibrium at close to neutral pH. Above pH 7.20, the deprotonated 1:2 complex, MA_2 is the dominant species present in solution. In alkaline pH, there is evidence for formation of hydroxo complexes, MA_2OH and $MA_2(OH)_2$ even though they have very low stability constants (Table 3). The total complexed Ni(II) percentage is also shown in Fig. 1 and it is evident that there is considerable uncomplexed

TABLE 2. Ligand and metal concentration variation (mol l^{-1}) during titration of the $Ni(II)/H_2NCH_2(CONHCH_2)_n$-CONHOH system

$C_{H_2NCH(CONHCH_2)_nCONHOH}$		$C_{Ni(II)}$ (M)
n = 1	n = 2	
6.12×10^{-3}	6.42×10^{-3}	2.00×10^{-4}
6.12×10^{-3}	6.42×10^{-3}	6.00×10^{-4}
6.12×10^{-3}	6.42×10^{-3}	12.00×10^{-4}
3.06×10^{-3}	3.21×10^{-3}	6.00×10^{-4}
9.18×10^{-3}	9.63×10^{-3}	6.00×10^{-4}

C_{NaOH} = 0.25 M; C_{HCl} = 0.02 M; C_{KCl} = 0.2 M. Temperature = 25 ± 0.1 °C; argon atmosphere; total volume = 50 ml.

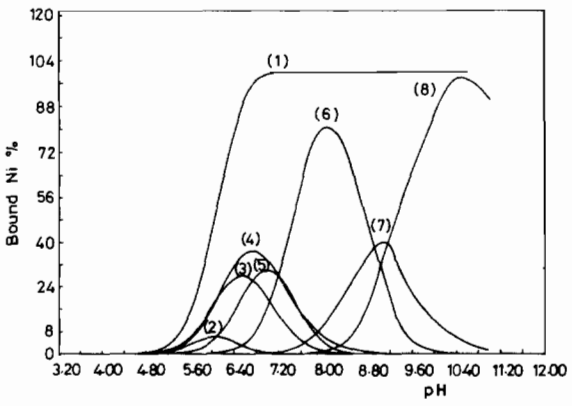

Fig. 1. Species distributions in the Ni(II)/diglycine hydroxamic acid system as a function of pH; $C_M = 10^{-4}$ M; C_A 6.120^{-3} M; (1) total bound Ni(II), (2) M_2A, (3) MA_2H_2, (4) MA, (5) MA_3H_3, (6) MA_2, (7) MA_2OH, (8) $MA_2(OH)_2$.

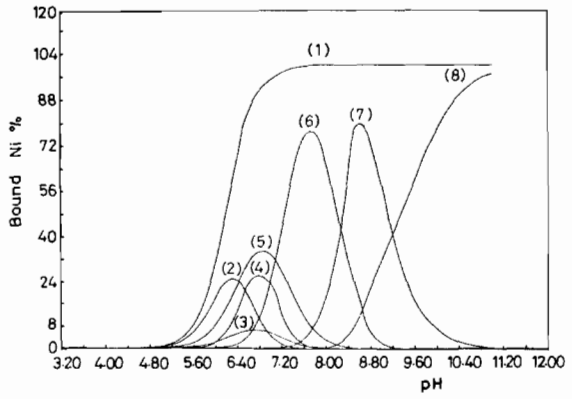

Fig. 2. Species distributions in the Ni(II)/triglycine hydroxamic acid system as a function of pH; $C_M = 6.0 \times 10^{-4}$ M; $C_A = 6.42 \times 10^{-3}$ M; (1) total bound Ni(II), (2) M_2A, (3) MA_2H_2, (4) $M_2A_3H_2$, (5) MA, (6) MA_2, (7) $MA_2(OH)_2$, (8) $MA_3(OH)_3$.

TABLE 3. Logarithmic stability constants ($\log \beta_{pqr}$) of complex species $M_pH_qA_r$ (M = Ni(II), A = $H_2NCH_2(CONHCH_2)_nCONHO$ (n = 0, 1, 2)) in 0.2 M KCl at 25 °C

p	q	r	$\log \beta_{pqr}$		
			n = 0	n = 1	n = 2
0	2	1	9.18	8.40	8.40
0	1	1	7.52	7.51	7.51
2	0	1		8.94	8.93
2	2	3			32.23
1	0	1	6.8	5.88	5.20
1	2	2		24.29	22.76
1	0	2	13.50	10.23	9.37
1	−1	2	4.24	1.32	
1	−2	2		−7.79	−7.18
1	3	3		36.11	
1	−3	3			−14.10

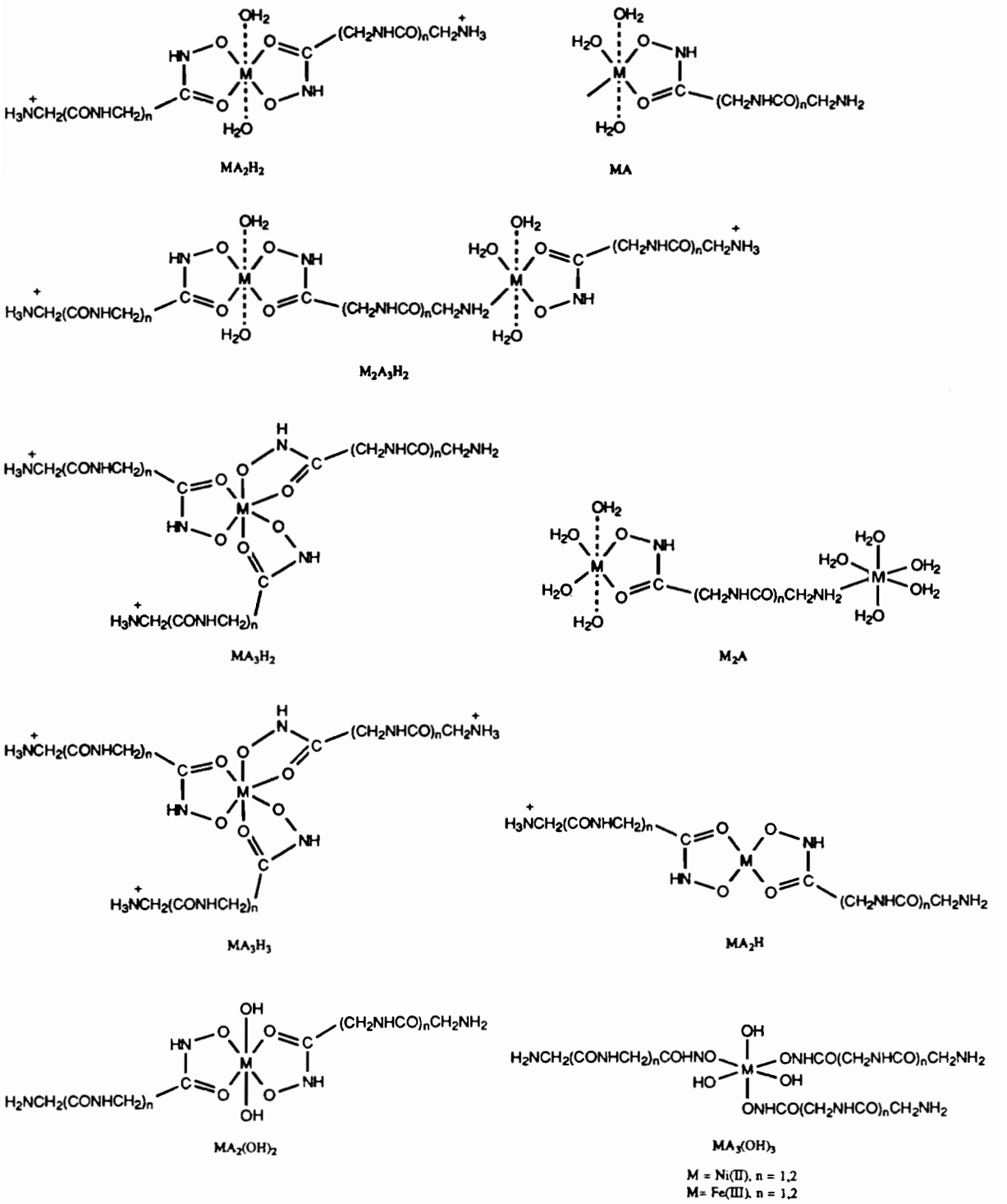

Fig. 3. Proposed structures for the complex species.

Ni(II) up to a pH of 6.50. $\delta H^+/\delta C_M$ as a function of pH (see 'Supplementary Material') shows that the molar proton liberation increases steadily from pH = ~5.0 to pH = ~8.0 where it reaches a maximum at a value of ~2 protons. The proposed structures for the major complex species are shown in Fig. 3.

The following species were detected for the Ni(II)/triglycine hydroxamic acid system (Fig. 2): M_2A, MA_2H_2, MA, $M_2A_3H_2$, MA_2H_{-2} and MA_3H_{-3}; see also 'Supplementary Material'. In the acidic region, two protonated complexes MA_2H_2 and $M_2A_3H_2$ and two deprotonated species M_2A and MA are formed;

no MA_3H_3 was detected. Above pH 7.20, the deprotonated 1:2 complex MA_2 is the dominant species present in solution as in the case of Ni(II)/glycylglycine hydroxamic acid system. In alkaline pH, there is evidence for formation of hydroxo complexes, MA_2 and $MA_3(OH)_3$. The proposed structures for the complex species are shown in Fig. 3.

Our results show that in the acidic pH range, dimeric species are formed in both the Ni(II)/GlyGlyHA and Ni(II)/GlyGlyGlyHA systems whereas no dinuclear species were detected in the Ni(II)/GlyHA system [3]; moreover, formation of dinuclear species (M_2A and $M_2A_3H_2$) is greater for triglycine hydroxamic acid than for glycylglycine hydroxamic acid where only a very small amount (max. 6% bound Ni%) of M_2A (and no $M_2A_3H_2$) was detected. One possible explanation is that the increase in distance between the amino group and CONHOH group decreases the steric hindrance thereby allowing the hydroxamic acid to coordinate in a tridentate manner and form dimers with the structures shown in Fig. 3. The generally low stability constant values quoted in Table 3 support coordination via the ketonic oxygen atom and the oxygen of the deprotonated NHO^- group.

The electronic absorption spectra of both the Ni(II)/GlyGlyHA system and the Ni(II)/GlyGlyGlyHA system were also recorded as a function of pH (see 'Supplementary Material'). No visible change was observed other than an increase of the extinction coefficient with pH, indicating increasing complexation.

Fe(III)/Hydroxamic Acid System

Fe(III) complexes

Both glycylglycine hydroxamic acid and triglycine hydroxamic acid form red 1:3 complexes, FeA_3 with Fe(III) at pH 8.00. The solid state magnetic moments of 5.27 and 5.28 BM at 20 °C for $(GlyGlyHA)_3Fe$, and their relative insensitivity to decreases in temperature (down to −180 °C) suggest that high spin weak field distorted octahedral complexes are formed.

Species distribution

The species distributions are shown as a function of pH in Figs. 4 and 5 and stability constants of the various species in Table 5 for the systems Fe(III)/$H_2NCH_2(CONHCH_2)_nCONHOH$ (n = 1, 2). Titration details are given in Table 4 (see also 'Supplementary Material').

The following species were detected for the Fe(III)/glycylglycine hydroxamic acid system (Fig. 4): M_2A, MA_2H_2, MA_3H_2, MA_2, MA_3, $MA_2(OH)_2$. In the acidic region, a protonated complex MA_2H_2 of a deprotonated complex M_2A are formed; the protonated complex MA_2H_2 predominates and no

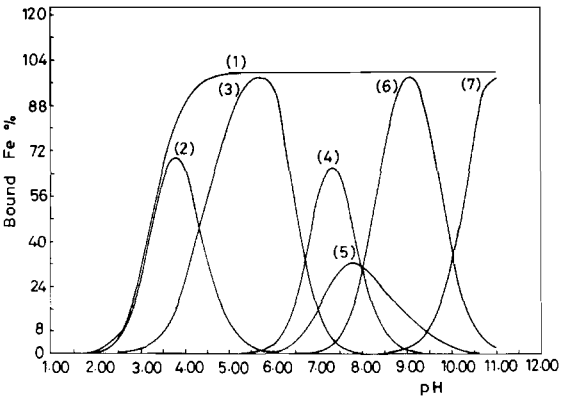

Fig. 4. Species distributions in the Fe(III)/diglycine hydroxamic acid system as a function of pH; $C_M = 3.13 \times 10^{-4}$ M; $C_A = 3.74 \times 10^{-3}$ M; (1) total bound Fe(III), (2) M_2A, (3) MA_2H_2, (4) MA_3H_2, (5) MA_2, (6) MA_3, (7) $MA_2(OH)_2$.

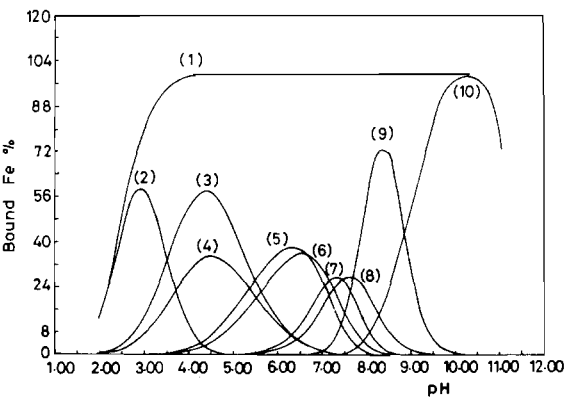

Fig. 5. Species distributions in the Fe(III)/triglycine hydroxamic acid system as a function of pH; $C_M = 5.01 \times 10^{-4}$ M; $C_A = 7.2 \times 10^{-3}$; (1) total bound Fe(III), (2) M_2A, (3) MA_2H_2, (4) MA, (5) MA_3H_3, (6) MA_2H, (7) MA_3H_2, (8) MA_2, (9) MA_3, (10) $MA_2(OH)_2$.

TABLE 4. Ligand and metal concentration variation (mol l^{-1}) during titration of the Fe(III)/$H_2NCH_2(CONHCH_2)_n$-CONHOH (n = 1, 2) system

$C_{H_2NCH(CONHCH)_n CONHOH}$		$C_{Fe(III)}$	
Fe(III)/$H_2NCH_2CONHCH_2$-CONHOH system		Fe(III)/$H_2NCH_2(CONHCH_2)_2$-CONHOH system	
C_A	$C_{Fe(III)}$	C_A	$C_{Fe(III)}$
3.74×10^{-3}	1.04×10^{-4}	7.20×10^{-3}	1.25×10^{-4}
3.74×10^{-3}	6.26×10^{-4}	7.20×10^{-3}	10.0×10^{-4}
1.87×10^{-3}	3.13×10^{-4}	3.60×10^{-3}	5.0×10^{-4}
3.74×10^{-3}	3.13×10^{-4}	7.20×10^{-3}	5.0×10^{-4}
5.61×10^{-3}	3.13×10^{-4}	10.80×10^{-3}	5.0×10^{-4}

MA was detected. The protonated MA_2H_2 species which is present at pH 5.70, is a precursor of the 1:2 complex and the protonated MA_3H_2 complex which

TABLE 5. Logarithmic stability constants ($\log \beta_{pqr}$) of complex species $M_pH_qA_r$ (M = Fe(III), A = $H_2NCH_2(CONH\text{-}CH_2)_nCONHO$ (n = 0, 1, 2) in 0.2 M KCl at 25 °C

p	q	r	$\log \beta_{pqr}$		
			$n = 0$	$n = 1$	$n = 2$
2	0	1		16.58	16.53
1	0	1	13.39		11.63
1	1	1	16.45		
1	2	2		30.16	29.99
1	1	2	27.56		24.37
1	0	2	21.87	18.90	17.14
1	−2	2		−3.99	0.668
1	3	3			42.75
1	2	3		36.23	35.72
1	0	3	26.50	22.22	20.50
1	1	3	33.86		
1	−2	3	6.89		

is the major complex species in equilibrium at close to neutral pH. In the alkaline region, the major peak belongs to the MA_3 species; above pH 9.0 there is evidence for the formation of the hydroxo complex $MA_2(OH)_2$. Figure 6 shows the proton liberation $\delta H^+/\delta C_M$ as a function of pH. The absence of any noticeable inflection up to pH 8.0 is consistent with a smooth transition from a 2:1 complex to a 1:3 complex and the decrease in $\delta H^+/\delta C_M$ after pH 8.0 is consistent with the formation of the hydroxo complex $MA_2(OH)_2$. The proposed structures for the complex species formed are shown in Fig. 3.

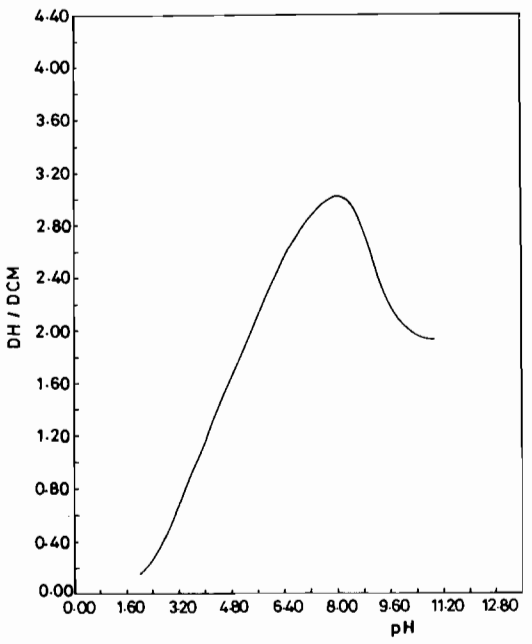

Fig. 6. Proton liberation $\delta H^+/\delta C_M$ for Fe/glygylglycine hydroxamic acid system as a function of pH.

The following species were detected for the Fe(III)triglycine hydroxamic acid system (Fig. 5): M_2A, MA_2H_2, MA, MA_3H_3, MA_2H, MA_3H_2, MA_2, MA_3, $MA_2(OH)_2$. In the acidic region, two protonated species MA_2H_2 and MA_3H_3 and two deprotonated species M_2A and MA are formed; two protonated species MA_2H and MA_3H_2 and the deprotonated MA_2 complex are detected at close-to-neutral pH. In the alkaline regions the major peak belongs to the neutral MA_3 complex. Above pH 8.00, there is evidence for formation of the hydroxo complex $MA_2(OH)_2$. The proposed structures for the complex species formed are shown in Fig. 3.

Visible Spectra

Visible spectra of the Fe(III)/glycylglycine hydroxamic acid and the Fe(III)/triglycine hydroxamic acid systems were recorded in the pH range 2.0–8.0. Both systems show absorption bands in the 380–430 nm region. Figure 7 shows the variation of λ of aqueous solutions of the Fe(III)/triglycine hydroxamic acid system with pH. The colour of the solution changes from wine red (pH 2.0 to 4.60) to orange–red at pH 5.20 and remains this colour up to pH 9.0. Only one absorption band was observed in the pH range 2.0 to 4.60 with λ_{max} changing from 465 (pH = 2.0) to 430 (pH 4.60) nm. At pH 5.20, a shoulder appears at λ_{max} 390 nm in addition to the shift of λ_{max} of the main absorption band to 425 nm. The λ_{max} of the main absorption band and the shoulder remains the same from pH 5.2 to 9.0 with the shoulder sharpen-

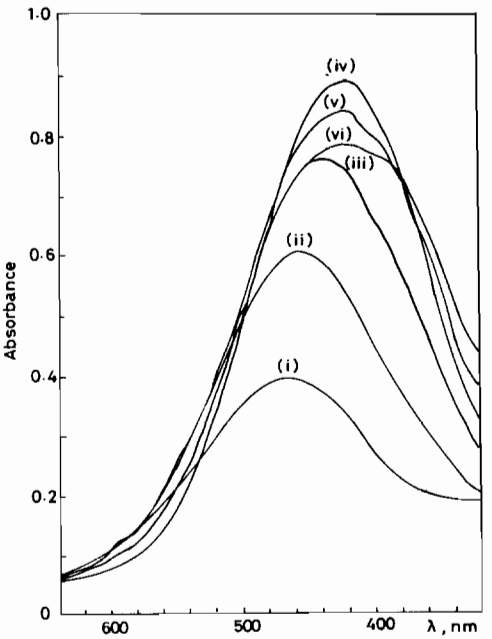

Fig. 7. The variation of λ_{max} in Fe/triglycine hydroxamic acid system with pH: (i) 2.0, (ii) 3.0, (iii) 4.40, (iv) 5.20, (v) 7.10, (vi) 8.0. [$C_{Fe(III)} = 1 \times 10^{-3}$ M; $C_A = 3 \times 10^{-3}$ M, 1 mm cells.]

ing with increase in pH (Fig. 6). The λ_{max} of the main absorption band increases rapidly from pH 2.0 to pH 6.0 and then decreases slightly with increase of pH. Since the species distribution curve (Fig. 5) of the Fe(III)/triglycine hydroxamic acid system suggests some aggregation of complex species at low pH values, the wine red colour may be due to species such as M_2A.

Conclusions

The peptide monohydroxamic acids, $H_2NCH_2(CONHCH_2)_nCONHOH$ ($n = 1, 2$) (**A**) have been shown to form stable complexes with Ni(II) and Fe(III) of the form NiA_2 and FeA_3 in which normal hydroxamate bonding involving the ketonic carbonyl and oxygen atom of the deprotonated NHO^- group occurs as evidenced by spectroscopic methods. Apparently, the length of the intervening peptide chain precludes stable coordination via the terminal amino group as previously observed in nickel(II) complexes of glycinehydroxamic acid [3]. However, solution studies primarily based on analytical potentiometry and electronic spectra/pH profiles indicate a wide range of complex species including a number of dimeric species. Further biological studies of these new complexes are in progress.

Supplementary Material

Sample titration curves for pK_a determinations and the proton liberation $\delta H^+/\delta C_A$ as a function of pH are available from the authors on request.

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