Studies on Transition-metal–Peptide Complexes. Part 8.† Parent and Mixed-ligand Complexes of Histidine-containing Dipeptides

Etelka Farkas, Imre Sóvágó, and Arthur Gergely *
Department of Inorganic and Analytical Chemistry, Lajos Kossuth University, H-4010 Debrecen, Hungary

The glycyl-L-histidine (GlyHis), L-histidylglycine (HisGly), and L-carnosine (ligands HA) parent complexes of cobalt(II), nickel(II), and zinc(II) were studied by pH-metry, spectrophotometry, and in part ^{13}C n.m.r. spectroscopy. All three metal ions were found to promote deprotonation of the peptide amide group of GlyHis. HisGly co-ordinates 'histidine-like' to the metal ions, *i.e.* without deprotonation of the peptide amide group. With carnosine, only nickel(II) induces deprotonation of this group. Studies were also made of mixed-ligand systems involving Gly, GlyGly, His, and occasionally 2,2′-bipyridyl as second ligand B, including those containing copper(II). Gly and GlyGly do not hinder the co-ordination of GlyHis *via* three nitrogens, and mixed-ligand complexes are therefore not present in detectable concentration. With His as ligand B, although mixed-ligand complexes are formed at comparable GlyHis and His concentrations, in the presence of an excess of His the parent complex $[M(HisO)_2]$ predominates. A mixed-ligand complex is obtained in significant amount with 2,2′-bipyridyl as ligand B. Appreciable formation of mixed-ligand complexes also occurs in the nickel(II)–carnosine–ligand B systems. For cobalt(II), however, practically only the parent complexes of ligands B are formed. The cobalt(II) complexes of all three dipeptides examined are able to take up molecular oxygen reversibly, the oxygen being released partially or almost completely. For GlyHis it is highly likely that the active complex is $[Co(AH_{-1})]$, while for HisGly and carnosine the presence of the bis complexes is presumed necessary for oxygen uptake.

Results on the complex-forming properties of glycyl-L-histidine (GlyHis), L-histidylglycine (HisGly), and β-alanyl-L-histidine (carnosine) have been surveyed by Sundberg and Martin.[1] The very varied investigations centred mainly on copper(II). It appears that the three ligands differ with respect to their deprotonation processes and the participation of the imidazole side-chain in the bonding (see also ref. 2 and the references therein).

The interactions of cobalt(II), nickel(II), and zinc(II) metal ions with GlyHis, HisGly, and carnosine (ligands HA) have also been studied.[3–8] Particular attention [9–12] was devoted to the cobalt(II) systems because of the reversible reaction of molecular oxygen with certain cobalt(II) complexes. Martin and co-workers [3–5] found that both nickel(II) and zinc(II) induce deprotonation of the peptide amide in the case of GlyHis, whereas only nickel(II) does so in the case of carnosine. This effect is even more marked in the cobalt(II)–GlyHis system,[5] but formation of the complex $[CoA_2H_{-1}]^-$ is hindered.

In the zinc(II)–GlyHis system, Agarwal and Perrin [7] also assumed the formation of dimeric complexes, while in the case of HisGly they concluded that 1 : 1 and 1 : 2 complexes are formed in which the co-ordination is histamine-like, *i.e.* the peptide amide does not take part in the bonding. This finding is in accordance with the conclusion of Yokoyama *et al.*[8] On the other hand, Harris and Martell [10] assumed deprotonation of the peptide amide even at about pH 7 in the 1 : 1 complex of cobalt(II) and HisGly.

In a study of the interaction with molecular oxygen, Harris and Martell [10] found that cobalt(II) forms a complex $[Co_2(AH_{-1})_2O_2(OH)]^-$ at pH 8 both with GlyHis and with HisGly. The peptide amide participates as the deprotonated form in this species. These two systems were also investigated by Michailidis and Martin.[11] In the absence of O_2, they excluded deprotonation of the peptide NH below pH 10 in the case of HisGly. In the presence of O_2 at a metal ion : ligand ratio of 1 : 2, they similarly assumed a dimeric intermediate, but this did not contain a hydroxy-bridge and the peptide amide group of the HisGly was deprotonated.

† Part 7 is ref. 2.

Brown and Antholine [12] carried out investigations at pH 7.2 on the cobalt(II)–carnosinec, -arnosine–histidine, and –carnosine–cysteine systems in the presence of O_2 at a wide range of metal ion : ligand ratios. In the presence of a large excess of ligand they assumed only the imidazole N^3 atom was co-ordinated. However, at a metal ion : ligand ratio of 1 : 1 they described a complex in which co-ordination of the deprotonated peptide N and the imidazole N^3 was highly probable, in addition to the amino-group. Further, in the presence of histidine or cysteine they concluded that stable mixed-ligand complexes are formed. They considered that the reversible interaction with oxygen occurs primarily in the mixed-ligand complexes, and that the parent complex containing carnosine undergoes very fast, irreversible oxidation.

It is clear from the foregoing that GlyHis, HisGly, and carnosine do indeed display different properties as regards the formation of complexes with cobalt(II), nickel(II), and zinc(II) ions. At the same time, it is not completely clear as to what identities and differences are manifested in the systems of these metal ions with different ligands. The possibility of the formation of bi- and multi-nuclear complexes is still disputed. Moreover, relatively few investigations have been performed on the mixed-ligand complexes of these ligands, and on the cobalt(II)–dipeptide–oxygen systems.

Accordingly, we have made an equilibrium and structural study of the cobalt(II), nickel(II), and zinc(II) complexes of GlyHis, HisGly, and carnosine. With the knowledge gained, studies were then made of the cobalt(II)–dipeptide–oxygen systems, and of the mixed-ligand complexes of Co^{II}, Ni^{II}, Cu^{II}, and Zn^{II} formed with histidine, glycine, glycylglycine, and occasionally 2,2′-bipyridyl as ligand B.

Experimental

Chemicals.—Chromatographically pure carnosine (BDH), glycyl-L-histidine hydrochloride (Sigma), and L-histidylglycine (Sigma) were used. The glycine, glycylglycine, histidine, and 2,2′-bipyridyl ligands B in the mixed-ligand complexes were Reanal products of the highest analytical purity.

The concentrations of the metal-ion stock solutions were checked gravimetrically *via* the quinolin-8-olates.

pH-*Potentiometric Measurements*.—Studies of the parent complexes were made at metal ion : ligand ratios of 1 : 1, 1 : 2, 1 : 3, 1 : 4, 1 : 5, and 1 : 6 in the metal-ion concentration range 2×10^{-3}—6×10^{-3} mol dm^{-3}, while those on the systems also containing ligand B involved the same metal-ion concentration range and metal ion : ligand A : ligand B ratios of 1 : 1 : 1, 1 : 1 : 2, or 1 : 2 : 1. For the systems containing copper(II), in this work only the mixed-ligand complexes of GlyHis and carnosine were studied, with 2,2′-bipyridyl and histidine as ligand B. The ratios corresponded to those above. Results for the parent copper(II) complexes were reported earlier.[2] All measurements were made at 25 °C, at an ionic strength of 0.2 mol dm^{-3} KCl. The pH-metric procedure employed and the manner of evaluating the data were described previously.[13]

The cobalt(II)–dipeptide–oxygen systems were investigated by both pH and pO_2 potentiometric measurements. In all cases the experiments were carried out at constant pH maintained with an ABUI3, a pHM 64, and a TTT60 pH-stat. Measurements were made at 25 °C, on samples containing 0.2 mol dm^{-3} KCl. The solutions were saturated with O_2. Under these conditions the O_2 concentration is 1.15×10^{-3} mol dm^{-3},[14] a value which we confirmed iodometrically.

In the cobalt(II)–GlyHis system, measurements were made at pH 9.2 at metal ion : ligand ratios of 1 : 1.2, 1 : 1.5, 1 : 2, 1 : 2.5, 1 : 3, and 1 : 5, and at pH 8.0 at ratios of 1 : 1, 1 : 2, and 1 : 5. The concentration of GlyHis was 2×10^{-3} or 4×10^{-3} mol dm^{-3}. In the cobalt(II)–HisGly–oxygen system, measurements were made at metal ion : dipeptide ratios of 1 : 1, 1 : 2, and 1 : 4, at pH 8, 9, and 9.5, and a metal-ion concentration of 1×10^{-3} mol dm^{-3}.

The cobalt(II)–carnosine–oxygen system was studied at metal ion : peptide ratios between 1 : 1 and 1 : 100, at pH 7.0, 7.5, 8.0, 8.2, and 8.8. At metal ion : ligand ratios above 1 : 10, measurements were also made at pH 10.5. The high ligand excess was justified by the low stabilities of these complexes.

The cobalt(II)–carnosine–histidine–oxygen system was examined using a metal-ion concentration of 6×10^{-3} mol dm^{-3} at cobalt(II) : carnosine : histidine ratios of 1 : 10 : 1 and 1 : 10 : 2.

Knowing the measured oxygen uptake, the nature of the species formed in the absence of O_2, and their stability constants, equilibrium constants were calculated for the peroxo-compounds assumed to be formed in the presence of O_2.

Spectral Studies.—In the case of the nickel(II)–carnosine system, measurements were made in the visible and near i.r. regions with a Beckman Acta MIV spectrophotometer, at a nickel(II) concentration of 0.01 mol dm^{-3} and metal ion : ligand ratios of 1 : 1, 1 : 2, and 1 : 5.

The ^{13}C n.m.r. studies on the zinc(II)–GlyHis system were made with a Brucker WP 200SY impulse Fourier-transform spectrometer. In the course of the recordings, 16 K data collection and complete proton decoupling were applied. The GlyHis concentration was 0.2 mol dm^{-3} in each case. Deuterium oxide was used as solvent, and also served as the heteronuclear lock. The samples contained dioxan at a concentration of 0.1—0.2 mol dm^{-3}. Accordingly, the chemical shifts in Table 2 refer to the ^{13}C n.m.r. signal of dioxan. All measurements were made at 35 ± 2 °C.

Results and Discussion

Parent Complexes.—GlyHis. For the titration of the 1 : 1 nickel(II)–, cobalt(II)–, and zinc(II)–GlyHis systems up to pH *ca.* 8, four equivalents of base are needed. This is in agreement with earlier findings [5,7,10] and with the result obtained for the copper(II)–GlyHis system,[2] and strongly suggests that the peptide amide is deprotonated in the 1 : 1 complex. As found by Morris and Martin,[4] at around pH 9 in the case of nickel(II) the colour of the solution changes to yellow, simultaneously with a new alkali-consuming process. With zinc(II) and cobalt(II), on the other hand, addition of more than four equivalents of base leads to formation of a precipitate. If the metal ion : ligand ratio is ≥1 : 2, the number of base equivalents consumed is higher than when the free ligand is titrated. On this basis it was assumed that, for all three metal ions, as for copper(II),[2] the complexes $[MAH_{-1}]$ and $[MA_2H_{-1}]^-$ are formed in which one of the peptide groups is deprotonated. [Brookes and Pettit [6] came to similar conclusions for the nickel(II)–GlyHis system.] In Table 1 the species formed in the range pH 4—9 are listed, together with the corresponding stability constants.

Table 1. Stability constants of cobalt(II), nickel(II), and zinc(II) complexes of GlyHis: $\beta_{pqr} = [M_pA_qH_r]/[M]^p[A]^q[H]^r$; 25 °C, I = 0.2 mol dm^{-3} KCl (Protonation constants of the ligand: 8.22, 14.99, and 17.50)

	Co^{II}	Ni^{II}	Zn^{II}
$\log \beta_{111}$	10.61	11.34	10.87
$\log \beta_{110}$	3.44	4.68	3.98
$\log \beta_{11-1}$	−3.96	−1.35	−2.75
$\log \beta_{120}$	6.57	9.64	8.03
$\log \beta_{12-1}$	−1.49	2.07	0.37
$\log \beta_{11-2}$	−15.45	—	−12.66
pK_{MA}^{MAH}	7.17	6.66	6.89
$pK_{MAH_{-1}}^{MA}$	7.40	6.03	6.73
$\log K_{MAH_{-1}}^{MA_2H_{-1}}$	2.47	3.42	3.12
$\log K_1/K_2$	0.31	−0.28	−0.07

Calculations with the complexes in Table 1 yielded the best fit to the titration data. In accordance with the assumption of Agarwal and Perrin,[7] we also carried out calculations which took account of the formation of binuclear complexes of zinc(II), but this led to a deterioration of the fitting.

The possibility of formation of binuclear complexes was studied further by ^{13}C n.m.r. measurements. In the concentrated solutions (≫100 mmol dm^{-3}) necessary for this, however, valid results could not be obtained because of precipitation in the range of deprotonation of the peptide NH. Thus, these measurements provided information merely on the initial stages of complex formation.

Chemical shift values relating to the processes of ligand deprotonation and complex formation are given in Table 2. The data indicate that H_2A^+ has a ^{13}C n.m.r. spectrum identical to that of 1 : 1 Zn^{2+}–GlyHis solution containing one equivalent of base. At pH <4, therefore, the metal ion–ligand interaction is negligible. A second equivalent of base results in chemical shifts in the signals of the imidazole and peptide carbonyl carbon atoms, indicating co-ordination of the imidazole N and the NH_2 and CO groups of the glycine moiety. This finding is supported by the pK_{MA}^{MAH} values in Table 1. For both zinc(II) and cobalt(II), these values are somewhat larger than the pK value (6.77) for the imidazole, but substantially smaller than that (8.22) for the amino group. The presence of metal ions generally results in a decrease in the protonation constants of the ligand. It may be presumed, therefore, that in the complexes $[MAH]^{2+}$ and $[MA]^+$ there exists an equilibrium between co-ordination *via* the amino- and carbonyl groups and that *via* the imidazole N^3 atom.

Table 2. Results of ^{13}C n.m.r. studies on the zinc(II)–GlyHis system (p.p.m. relative to 1,4-dioxan)

System	Equivalents of base	Species	CH_2(His)	CH_2(Gly)	CH(His)	C^5	C^4	C^2	CO(pept)	COO^-
GlyHis	0	H_3A^{2+}	−40.31	−26.03	−14.54	50.68	61.92	67.07	100.39	106.37
	1	H_2A^+	−39.32	−25.92	−12.33	50.26	63.16	66.84	99.91	109.30
	2	HA	−37.68	−24.55	−11.49	51.31	66.06	68.95	—	110.57
	3	A^-	−37.30	−22.49	−11.64	51.75	66.51	69.56	108.20	111.10
Zn^{II}–GlyHis (1 : 1)	1	$Zn^{2+} + H_2A^+$	−39.48	−25.97	−12.49	50.51	62.91	66.99	100.15	109.28
	2	$[Zn(HA)]^{2+}$ / $[ZnA]^+$	−39.02	−25.88	−11.90	53.10	63.64	69.41	102.70	110.05

Table 3. Stability constants of cobalt(II), nickel(II), and zinc(II) complexes of HisGly at 25 °C and I = 0.2 mol dm^{-3} KCl (Protonation constants of the ligand: 7.58, 13.53, and 16.49)

	Co^{II}	Ni^{II}	Zn^{II}
$\log \beta_{110}$	5.22	6.81	5.07
$\log \beta_{120}$	9.28	12.30	9.54
$\log K_1/K_2$	1.16	1.32	0.63

Table 4. Stability constants of cobalt(II), nickel(II), and zinc(II) complexes of carnosine at 25 °C and I = 0.2 mol dm^{-3} KCl (Protonation constants of the ligand: 9.39, 16.23, and 18.76)

	Co^{II}	Ni^{II}	Zn^{II}
$\log \beta_{111}$	11.48	12.11	11.62
$\log \beta_{110}$	2.85	4.61	4.00
$\log \beta_{11-1}$	−6.10	−3.22	—
$\log \beta_{122}$	21.91	—	—
$\log \beta_{12-1}$	—	1.06	—
pK_{MA}^{MAH}	8.63	7.50	7.62
pK_{MAH-1}^{MA}	8.95	7.83	—

The data in Table 1 demonstrate that, in the physiological pH range, deprotonation of the peptide amide is promoted by the metal ions in the sequence $Ni^{II} > Zn^{II} > Co^{II}$. This process cannot be suppressed by an excess of ligand, in spite of the fact that $\log K_1/K_2$ is very small, or even negative in the nickel(II) and zinc(II) complexes. This apparent contradiction can be explained if, as for copper(II),[2] it is assumed that the complexes $[MA_2]$ in fact have compositions $[M(AH_{-1})(HA)]$, *i.e.* bis complexes which do not involve deprotonation of the peptide amide are not formed or at least are present in very low concentration. It also emerges from Table 1 that $\log K_{MAH_{-1}}^{MA_2H_{-1}}$ corresponding to the co-ordination of the second ligand, is relatively low for all of the metals. This is particularly the case for cobalt(II)–GlyHis. The low value of the stability constant may be explained [5] if the formation of $[CoA_2H_{-1}]^-$ is hindered.

HisGly. Contradictory data concerning complexes of this ligand are available. In the presence of cobalt(II), Michailidis and Martin [11] exclude deprotonation of the peptide amide below pH 10, whereas Harris and Martell [10] consider it possible even around the physiological pH. In the case of nickel(II), Brookes and Pettit [6] assume histamine-like co-ordination and postulate only the complexes $[NiA]^+$ and $[NiA_2]$; in addition, they exclude deprotonation of the peptide amide up to pH 9. Yokoyama *et al.*[8] carried out measurements at a metal ion : ligand ratio of 1 : 2, and described the cobalt(II)–, nickel(II)–, and zinc(II)–HisGly systems assuming the formation of complexes $[MA]^+$ and $[MA_2]$.

In the present work it was found that, in contrast to the copper(II)–HisGly system,[2] in 1 : 1 solutions, precipitation of metal hydroxides occurred after the addition of more than two equivalents of base. Thus, deprotonation (and co-ordination) of the peptide amide does not occur below pH *ca.* 8. At metal ion : ligand ratios of $\geq 1 : 2$, however, the titration data can be fitted well by the assumption of bis complexes $[MA_2]$. In these systems, therefore, it is not necessary to consider deprotonation of the peptide NH group of HisGly. The stability constants of the complexes formed are given in Table 3, together with the $\log K_1/K_2$ values.

The data in Table 3 are strongly suggestive of ' histidine-like ' co-ordination of HisGly with all three metal ions. The constants approximate well to the corresponding data for the histidine complexes.[6,15] In the case of HisGly, therefore, co-ordination of the amino group and of the imidazole N^3 is highly probable for all three metal ions examined.

Carnosine. Apart from the early investigations by Martin and Edsall,[3] few data are available on the cobalt(II), nickel(II), and zinc(II) complexes of this dipeptide. Brookes and Pettit [6] reported findings on the interaction of nickel(II) with carnosine, as did Agarwal and Perrin [7] on those of cobalt(II) and zinc(II).

In our experiments the 1 : 1 nickel(II)–carnosine system was found to consume four equivalents of base up to pH *ca.* 9.2; as for nickel(II)–GlyHis (but at a higher pH), this suggests deprotonation of the peptide amide. On addition of more base, however, a precipitate occurs. In an attempt to confirm the deprotonation process, spectrophotometric measurements were carried out in the near-i.r. region. The following band maxima were observed. Formation of a hydroxo-complex

pH	5.5	6.3	6.7	7.3	7.9	8.5
$\lambda_{max.}$/nm	940	925	915	910	910	910

would not result in the shift in $\lambda_{max.}$ towards lower wavelengths, and we therefore consider our former assumption regarding the deprotonation of the peptide amide to be confirmed.

In the presence of an excess of ligand for all three metal ions, in accordance with earlier studies,[6,7] definite conclusions could not be reached from pH-metric measurements regarding the formation of complexes with a composition higher than 1 : 1. This may be explained in that these can be formed in measurable concentration only at a relatively high pH, and may not be accompanied by an appreciable pH effect. Nevertheless, from chemical considerations and a comparison with the results for GlyHis, the formation of the complex $[NiA_2H_{-1}]^-$ may be possible.

The cobalt(II), and particularly the zinc(II) system, can be studied only over a very narrow pH interval. The extent of complex formation is appreciable only around pH 6, and

Table 5. Stability constants of mixed-ligand complexes, metal ion–GlyHis–ligand B, at 25 °C and I = 0.2 mol dm^{-3} KCl; $\beta_{pqrs} = [M_pA_qB_rH_s]/[M]^p[A]^q[B]^r[H]^s$

Metal ion	Ligand B	log $\beta_{1\,1\,1\,1}$	log $\beta_{1\,1\,1\,0}$	log $\beta_{1\,1\,1\,-1}$	pK^{MABH}_{MAB}	$pK^{MAB}_{MABH_{-1}}$
Co^{II}	Histidine	16.95	9.63	0.90	7.28	8.73
Ni^{II}	Histidine	19.57	12.77	4.52	6.80	8.25
Cu^{II}	Histidine	20.93	—	8.19	—	—
Cu^{II}	2,2′-Bipyridyl	20.93	15.20	8.40	5.73	6.80
Zn^{II}	Histidine	17.62	10.32	1.92	7.30	8.40

above pH *ca.* 7.5 a precipitate separates from a zinc(II) solution even in the presence of excess of ligand.

Table 4 shows the obtained stability constants and some derived data. (The complexes are of fairly low stability, and hence the error in the values is comparatively high.) The small stability constants for the cobalt(II)–carnosine interaction and the high pK values for the complexes (the absolute values of the constants do not serve as direct evidence) primarily indicate the formation of hydroxo-complexes, and not deprotonation of the peptide amide. In contrast to the glycyl moiety, therefore, the β-alanyl moiety promotes deprotonation of the amide group to a much lower extent.

Mixed-ligand Complexes.—The mixed systems of GlyHis and HisGly with cobalt(II) or zinc(II) and histidine were studied by Agarwal and Perrin,[7] who found that the mixed-ligand complex formation almost corresponds to that expected statistically. Since the nature of ligand B may have a significant effect on the ability of the dipeptides to form complexes, and also on the deprotonation conditions, we have extended such investigations to a wider range of ligands B. Experiments have also been carried out on the copper(II)–GlyHis and –carnosine mixed-ligand systems. In the case of GlyHis, the compounds glycine, glycylglycine, and histidine served as ligand B, as did 2,2′-bipyridyl for copper(II). 2,2′-Bipyridyl was likewise used instead of glycine for the mixed-ligand complexes of copper(II) and zinc(II) with carnosine.

The protonation constants of 2,2′-bipyridyl and the stability constants of its copper(II) and zinc(II) complexes were taken from the literature.[16] The stability constants of the parent complexes of histidine with copper(II), nickel(II), and zinc(II), and of glycine and glycylglycine with nickel(II) and zinc(II), were determined earlier.[15,17] Those of the glycine, glycylglycine, and histidine complexes of cobalt(II) were obtained in this work.*

The experimental findings for the metal ion–GlyHis–ligand B mixed systems were as follows. Even in the case of an excess of ligand, glycylglycine is scarcely able to form a parent complex, and no mixed-ligand complex is formed at all. A mixed-ligand complex is not formed in the case of glycine either, although [MB_2] is present in measurable concentration besides [MAH_{-1}].

The non-formation of mixed-ligand complexes in the cases of glycylglycine and glycine can be explained in that these ligands are not able to suppress deprotonation of the amide group of GlyHis. Thus, they do not influence the stable bonding between the amide nitrogen and the metal ions. The situation is essentially different if histidine or 2,2′-bipyridyl is used as ligand B. Mixed-ligand complexes of composition [MABH], [MAB], and [$MABH_{-1}$] (overall charges are omitted as they depend on the type of ligand B) are then formed in measurable concentrations. The relevant constants are listed in Table 5.

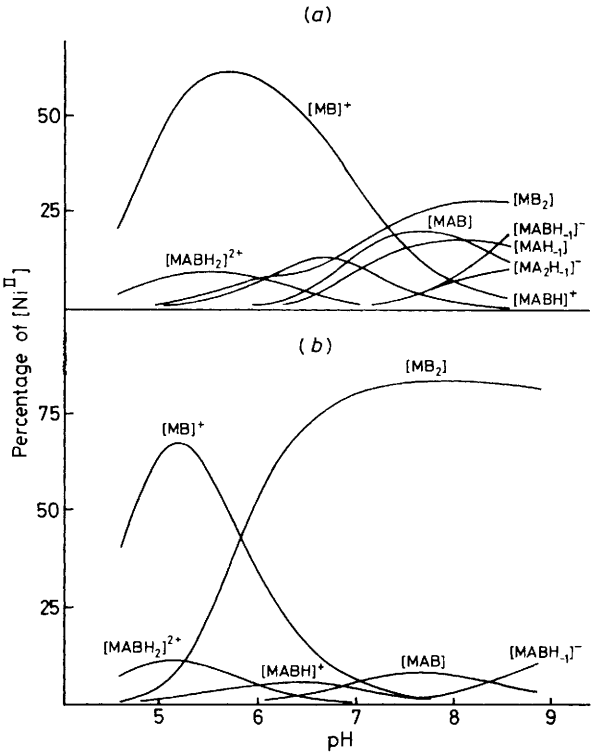

Figure 1. Concentration distribution of complexes formed at ratios of 1 : 1 : 1 and 1 : 1 : 2 in the nickel(II)–GlyHis–histidine system: (*a*) $c_{Ni} = c_{GlyHis} = c_{His} = 2 \times 10^{-3}$ mol dm^{-3}; (*b*) $c_{Ni} = c_{GlyHis} = 2 \times 10^{-3}$, $c_{His} = 4 \times 10^{-3}$ mol dm^{-3}

The values for cobalt(II) in this Table are similar to those reported by Agarwal and Perrin,[7] but those for zinc(II) are only partially so. The explanation for this may be that the previous authors assumed formation of the binuclear zinc(II)–GlyHis parent complex in their calculations, whereas we could not confirm its existence (see above).

The distribution curves of the complexes formed at the various metal ion : ligand ratios in the nickel(II)–GlyHis–histidine system are presented in Figure 1. From these plots it seems that the formation of mixed-ligand complexes is not significant for histidine either. Even, an excess of histidine suppresses almost completely the co-ordination of GlyHis, and the [NiB_2] histidine complex is formed predominantly.

The results on the copper(II)–GlyHis–2,2′-bipyridyl system show that of ligands B 2,2′-bipyridyl has the strongest tendency to form mixed-ligand complexes. This may presumably be explained in that, as in other systems,[18] the 2,2′-bipyridyl occupies one equatorial and one axial site. In

* For cobalt(II)–glycine, log $\beta_{CoA} = 4.64$, log $\beta_{CoA_2} = 8.46$; for cobalt(II)–glycylglycine, log $\beta_{CoA} = 2.90$, log $\beta_{CoA_2} = 5.31$; and for cobalt(II)–histidine, log $\beta_{CoAH} = 10.98$, log $\beta_{CoA} = 6.76$, log $\beta_{CoA_2H} = 17.36$, and log $\beta_{CoA_2} = 12.18$.

Table 6. Stability constants of mixed-ligand complexes of carnosine at 25 °C and I = 0.2 mol dm^{-3} KCl

Metal ion	Ligand B	log $\beta_{1\,1\,1}$	log $\beta_{1\,1\,0}$	log $\beta_{1\,1\,-1}$	pK^{MABH}_{MAB}	$pK^{MAB}_{MABH_{-1}}$
Co^{II}	Glycine	16.10	7.3	−2.0	8.8	—
	Glycylglycine	14.50	6.2	−2.8	8.3	—
	Histidine	18.23	9.23	—	9.0	—
Ni^{II}	Glycine	17.61	9.73	0.88	7.88	8.85
	Glycylglycine	16.02	8.03	−0.24	7.99	8.27
	Histidine	20.12	12.03	2.48	8.09	9.55
Cu^{II}	Histidine	23.38	17.28	7.86	6.10	9.42
	2,2′-Bipyridyl	21.52	14.53	5.28	6.99	8.29
Zn^{II}	Histidine	17.62		—		
	2,2′-Bipyridyl	16.51	8.60	—	7.91	

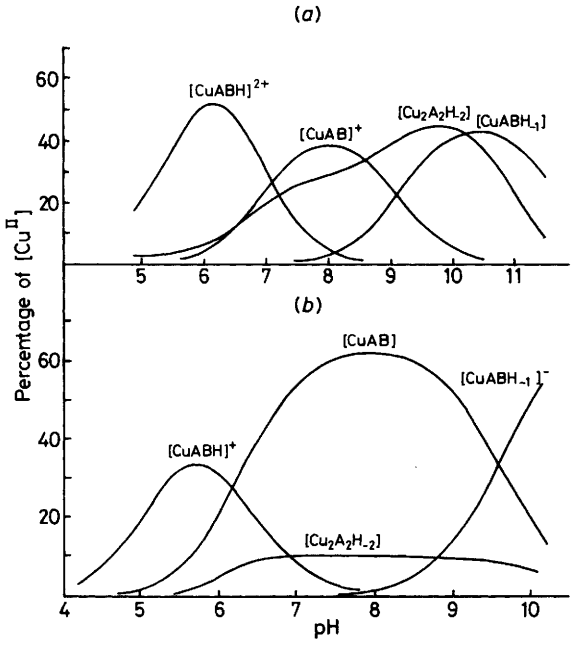

Figure 2. Concentrations of mixed-ligand complexes and multinuclear complexes formed in the copper(II)–L-carnosine–2,2′-bipyridyl (*a*) and in the copper(II)–L-carnosine–L-histidine (*b*) systems as a function of pH. $c_{Cu} = c_A = c_B = 2 \times 10^{-3}$ mol dm^{-3}

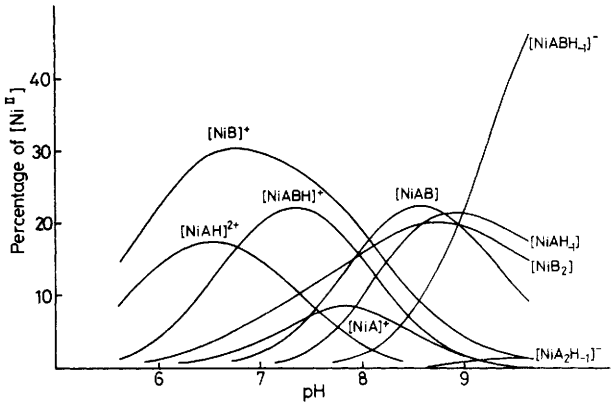

Figure 3. Concentration distribution of complexes formed at a ratio of 1 : 1 : 1 in the nickel(II)–carnosine–glycine system. $c_{Ni} = c_{carnosine} = c_{Gly} = 6 \times 10^{-3}$ mol dm^{-3}

this way a mixed-ligand complex [CuABH$_{-1}$] may be formed, in which ligand B does not hinder the stable co-ordination of GlyHis to yield [CuAH$_{-1}$].

Table 6 gives constants for the mixed-ligand complexes formed with carnosine. The data reveal that in both the copper(II)–carnosine–2,2′-bipyridyl and copper(II)–carnosine–L-histidine systems a considerable degree of mixed-ligand complex formation occurs. However, the amounts of such complexes formed differ appreciably from those of the carnosine parent complexes.

Our results for the copper(II)–carnosine system were discussed in detail earlier.[2] Here we state only that the peptide group is deprotonated below pH *ca.* 6, and subsequently merely the dimer [Cu$_2$A$_2$H$_{-2}$] is formed up to pH *ca.* 10. No 1 : 2 complexes are formed in measurable concentration up to a metal ion : ligand ratio of 1 : 5.

In the absence of 1 : 2 parent complexes, statistical constants for the mixed-ligand complexes cannot be calculated; thus, information on the extents of mixed-ligand complex formation must be obtained from the concentration distribution. The concentrations of the dimeric and the mixed-ligand complexes are shown in Figure 2, from which it may be seen that 2,2′-bipyridyl is only partially able to suppress the formation of [Cu$_2$A$_2$H$_{-2}$]. In the presence of L-histidine, however, the dimer is hardly formed. In neutral or weakly alkaline media, [CuABH]$^+$ and [CuAB] are present in the copper(II)–carnosine–L-histidine system, whereas [CuABH$_{-1}$]$^-$ begins to form only at comparatively high pH. This means that the stable histidine-type co-ordination considerably suppresses deprotonation of the peptide NH.

In the case of nickel(II)–carnosine, mixed-ligand complexes are formed with all three ligands B. The concentration distribution of the complexes formed in the nickel(II)–carnosine–glycine system is depicted in Figure 3.

Formation of mixed-ligand complexes is substantially less favoured in the cobalt(II)–carnosine–ligand B systems than for nickel(II). This is illustrated in Figure 4 for the cobalt(II)–carnosine–glycine system. It is clear that only the mixed-ligand complex [CoABH]$^+$ is formed in significant concentration.

When the pK^{NiABH}_{NiAB} and $pK^{NiAB}_{NiABH_{-1}}$ values in Tables 5 and 6 are compared with the corresponding pK values for the parent complexes in Tables 1 and 4, it is clear that the deprotonation processes occur at a higher pH interval for the

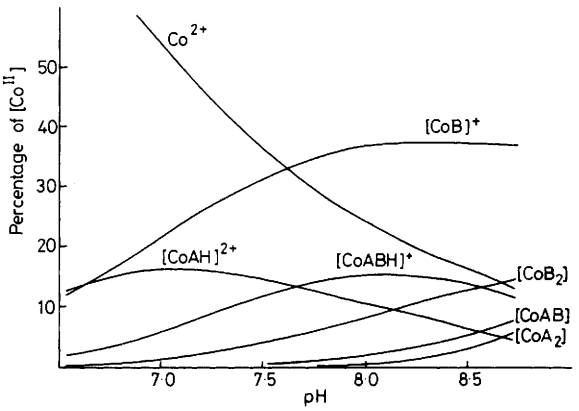

Figure 4. Concentration distribution of complexes formed at a ratio of 1 : 1 : 1 in the cobalt(II)–carnosine–glycine system. $c_{Co} = c_{carnosine} = c_{Gly} = 6 \times 10^{-3}$ mol dm^{-3}

mixed-ligand complexes. The difference is considerable in the mixed-ligand complexes of glycine, and particularly histidine, as ligand B. One possible explanation of these experimental results is as follows. The tendency towards the formation of two chelate rings in the parent complexes may lead to deprotonation of the amino- and peptide amide groups at lower and similar pH values. In the mixed-ligand complexes, however, since glycine and histidine occupy two and three co-ordination sites, respectively, co-ordination of the peptides as in the parent complexes is hindered. Accordingly, the corresponding deprotonation processes occur at higher pH than for the parent complexes, and are well separated. However, this does not provide a satisfactory explanation for the data for the nickel(II)–carnosine–glycylglycine system, where although the deprotonation processes $[NiABH]^+ \rightleftharpoons [NiAB] + H^+$ and $[NiAB] \rightleftharpoons [NiABH_{-1}]^- + H^+$ take place at a slightly higher pH than for the parent complexes, they also overlap one another. Thus, the relevant constants do not even permit a clear-cut decision as to whether the peptide amide group of the glycylglycine or the carnosine is deprotonated. Further, prior to deprotonation of the peptide amide group, glycylglycine forms a stable bond only *via* the amino-group, and the peptide CO–nickel(II) bond is less stable. Consequently, it may be assumed that, in the case of glycylglycine, co-ordination of carnosine as in the parent complex is less hindered.

According to the experimental findings outlined above, the formation of mixed-ligand complexes is not favoured for GlyHis with all of the metal ions studied, and this is also the case in the cobalt(II)–carnosine system. Glycine and glycylglycine do not influence the co-ordination of GlyHis *via* three nitrogens. Histidine, however, suppresses considerably the co-ordination of GlyHis, and that of carnosine practically completely.

We examined the zinc(II)–carnosine–histidine and the zinc(II)–carnosine–2,2′-bipyridyl systems up to pH *ca.* 11. Compared to the species in Table 4, a zinc(II) parent complex of different composition could not be identified, even at higher pH. Nor was a mixed-ligand complex in which deprotonation of the peptide group of carnosine could have been assumed.

Cobalt(II)–Dipeptide–Oxygen Systems.—These systems were studied at constant pH by following the decrease in oxygen concentration to the achievement of equilibrium. Subsequently, acid was added to these basic solutions and the quantity of O_2 evolved was measured. The aim of the measurements was to obtain new information both on the complex formed upon O_2 uptake, and on deprotonation of the peptide amide.

We set out from the ' 3N rule '.[19] According to this, only those cobalt(II) complexes in which at least three nitrogen-donor atoms are present in the co-ordination sphere are capable of reversible O_2 uptake. The previously discussed equilibrium studies carried out with the exclusion of oxygen showed that, in the case of cobalt(II)–GlyHis, with deprotonation of the peptide amide this rule holds for the 1 : 1 complex, but in the cases of HisGly and carnosine it holds only for the 1 : 2 complexes formed in the presence of excess of ligand. At the same time, the appearance of the yellowish brown colour indicative of formation of the binuclear peroxo-complex [10] was observed in the 1 : 1 samples for all three systems. However, substantial differences were found in the lifetimes of these complexes, and the quantity of O_2 taken up was a function of the metal ion : ligand ratio and of the pH.

The results obtained for the cobalt(II) complexes are summarized below.

GlyHis. Deprotonation of the peptide amide in the absence of O_2 is complete at pH *ca.* 9 in the cobalt(II)–GlyHis complex (see above). Hence, the measurements were made at pH 9.2. In the interests of comparison with previous results [10,20] some studies were also carried out at pH 8.

It was found that equilibrium was reached very quickly at at pH 9.2, whereas at pH 8 there was still a slow O_2 uptake even after 1.5—2 min. This difference might be ascribed to the fact that at pH 8 the complex taking up O_2 is formed with a continuous shift of the equilibrium. In each case the effect measured up to attainment of equilibrium depended only on the cobalt(II) concentration, and not on the metal ion : ligand ratio. This supports the earlier results that the complex active in the reversible O_2 uptake is $[CoAH_{-1}]$,[10] and that the formation of $[CoA_2H_{-1}]^-$ can be neglected.[5] At a metal ion : ligand ratio of 1 : 1, 4.5 equivalents of base were consumed. This is very probably due to the formation of the complex $[Co_2(AH_{-1})_2O_2(OH)]^-$ assumed previously.[10] For this species the experimental data gave a value of $\log K = -1.6$, where $K = [Co_2(AH_{-1})_2O_2(OH)^-][H^+]/[CoAH_{-1}]^2[O_2]$. If the solutions are acidified to pH *ca.* 4 after some minutes, the complex gives off somewhat more than 90% of the O_2 taken up. This points to a fairly long half-life. (Michailidis and Martin [11] stated that the half-life of the peroxo-compound is *ca.* 7 h.)

HisGly. In order to avoid precipitation, measurements were made at pH 8 at a metal ion : ligand ratio of 1 : 1, and at pH 9 and 9.5 in the presence of excess of ligand. Formation of the complex $[CoA_2]$ was then complete.

In the absence of O_2, deprotonation of the peptide amide group of HisGly does not occur in the presence of cobalt(II), and thus co-ordination of merely two nitrogens may be assumed in the 1 : 1 complex. Consequently, with regard to the ' 3N rule,' O_2 uptake is to be expected only for the 1 : 2 complex of cobalt(II)–HisGly. Experimentally, however, there is also a little O_2 consumption in 1 : 1 solutions. This may be explained in terms of the fact that in this case too the active complex is in fact $[Co(HisGlyO)_2]$. Bearing in mind the relatively low value of $\log K_1/K_2$ (see Table 3), this complex may be formed in low concentration even in the 1 : 1 samples. Hence, when O_2 is passed into the system, the equilibrium shifts increasingly in the direction of $[CoA_2]$ formation. This assumption is supported by a comparison of the results from measurements at various metal ion : ligand ratios and at various pH values: the attainment of equilibrium is substantially slower at a ratio of 1 : 1 than at higher metal ion : ligand ratios, *i.e.* the measured O_2 consumption is also essentially lower.

A detailed elucidation of the stoicheiometric conditions is made difficult by the fact that the half-life of the cobalt(II)–

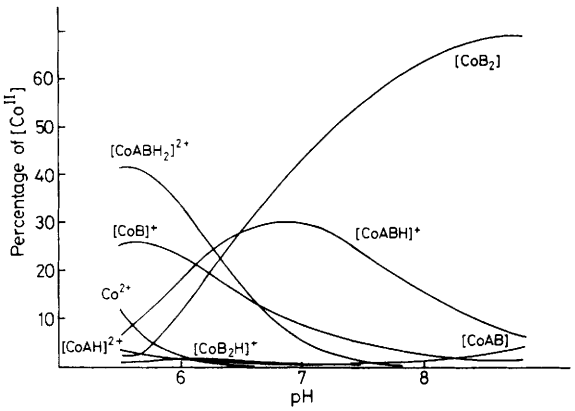

Figure 5. Concentration distribution of complexes formed at a ratio of 1 : 10 : 2 in the cobalt(II)–carnosine–histidine system. $c_{Co} = 6 \times 10^{-3}$, $c_{carnosine} = 6 \times 10^{-2}$, and $c_{His} = 1.2 \times 10^{-2}$ mol dm^{-3}

HisGly peroxo-complex is short.[11] However, from the consumption of base it may be concluded that it is necessary to consider deprotonation of the peptide amide in the complex formed in the presence of O_2. It is also highly likely that the deprotonation of one peptide amide corresponds to one cobalt(II). However, the participation of a hydroxo-bridge in the transition-state complex cannot be verified from the base consumption. If the ' histidine-analogue ' composition $[(CoA_2)_2O_2]$ is assumed for the complex formed initially, the constant determined from the experimental results (log K = 6.4 ± 0.1) agrees to a very good approximation with the value reported by Martell and co-workers [21] for histidine.

Carnosine. In the case of carnosine, measurements were made at metal ion : ligand ratios of 1 : 1—1 : 100, and in the range pH 7—10.5. The formation of a peroxo-compound of short half-life can be assumed, its stability appearing to increase as the pH is raised. At pH 7 there is scarcely any O_2 uptake, but with increasing pH the quantity of O_2 consumed becomes more appreciable. If the solution is acidified 3—4 min after initiation of the reaction, the complex does not release the O_2 taken up at pH ≤ 9; at pH 10.5, however, 30% of it can be recovered. From measurements in the absence of O_2, deprotonation of the peptide amide could not be verified for carnosine either. In the presence of O_2, on the other hand, it seems that deprotonation of the peptide amide must be considered. Conclusions as to the participation of a hydroxyl group in the transition-state complex cannot be drawn from the number of base equivalents consumed. If calculations are made on the basis of unit metal-ion concentration, at a given pH, the measured O_2 consumption is also a function of the metal ion : ligand ratio. The latter observation may be explained by the low stability of the cobalt(II)–carnosine complexes (see Table 4), insofar as an increase in the metal ion–ligand ratio increases the proportion of the oxygen-sensitive complex. In the case of the oxygen-sensitive complex, here too one may resort to the conclusions made for HisGly. Thus, it seems most probable that the process begins with O_2 uptake by the 1 : 2 complex, in the course of which the peptide amide also undergoes deprotonation. Subsequently, simultaneous co-ordination of the amino-group, the peptide N, and the imidazole N^3 atom is assumed.

With regard to earlier findings on carnosine,[12] experiments were also made on the cobalt(II)–carnosine–histidine–oxygen system. The equilibrium results in the absence of O_2 and showed that there is no appreciable formation of mixed-ligand complexes in the cobalt(II)–carnosine–histidine system. Figure 5 demonstrates that if the histidine concentration is twice the cobalt(II) concentration, practically only $[Co(HisO)_2]$ is formed, even when carnosine is present in a several-fold excess.

In agreement with Brown and Antholine,[12] a reversible process must be taken into account above pH 8 in the presence of O_2 in the cobalt(II)–carnosine–histidine system. It is clear from Figure 5, however, that the complex $[Co(HisO)_2]$ does indeed take up O_2. Actually, the stability constant (log K = 6.5 ± 0.1) calculated for the complex $[\{Co(HisO)_2\}_2O_2]$ from the experimental data agrees, here too, within error with the value reported for the cobalt(II)–histidine system.[21]

Acknowledgements

The authors express their thanks to Ferenc Matyuska for his experimental work and for his active participation in certain phases of the evaluation.

References

1 R. J. Sundberg and R. B. Martin, *Chem. Rev.*, 1974, **74**, 471 and refs. therein.
2 Part 7, I. Sóvágó, E. Farkas, and A. Gergely, *J. Chem. Soc., Dalton Trans.*, 1982, 2159.
3 R. B. Martin and J. T. Edsall, *J. Am. Chem. Soc.*, 1960, **82**, 1107.
4 P. J. Morris and R. B. Martin, *J. Inorg. Nucl. Chem.*, 1971, **33**, 2918.
5 L. G. Stadtherr and R. B. Martin, *Inorg. Chem.*, 1973, **12**, 1810.
6 G. Brookes and L. D. Pettit, *J. Chem. Soc., Dalton Trans.*, 1975, 2112 and refs. therein.
7 R. P. Agarwal and D. Perrin, *J. Chem. Soc., Dalton Trans.*, 1975, 1045.
8 A. Yokoyama, H. Aiba, and H. Tanaka, *Bull. Chem. Soc. Jpn.*, 1974, **47**, 112.
9 R. G. Wilkins, *Adv. Chem. Ser.*, 1971, **100**, 111.
10 W. R. Harris and A. E. Martell, *J. Am. Chem. Soc.*, 1977, **99**, 6746.
11 M. S. Michailidis and R. B. Martin, *J. Am. Chem. Soc.*, 1969, **91**, 4683.
12 C. E. Brown and W. E. Antholine, *Biochem. Biophys. Res., Commun.*, 1979, **88**, 529.
13 I. Nagypál, A. Gergely, and E. Farkas, *J. Inorg. Nucl. Chem.*, 1974, **36**, 699.
14 ' Solubilities of Inorganic and Metal Organic Compounds,' 4th edn., vol. 2, ed. W. F. Linke, Amer. Chem. Soc., 1965, p. 1228.
15 I. Sóvágó, T. Kiss, and A. Gergely, *J. Chem. Soc., Dalton Trans.*, 1978, 964.
16 G. Anderegg, *Helv. Chim. Acta*, 1963, **46**, 2397.
17 E. Farkas, B. Beke, and A. Gergely, *Magy. Kem. Foly.*, 1980, **86**, 345.
18 M. C. Lim, E. Sinn, and R. B. Martin, *Inorg. Chem.*, 1976, **15**, 807.
19 S. Fallab, *Angew. Chem., Int. Ed. Engl.*, 1967, **6**, 496.
20 R. D. Gillard and A. Spencer, *J. Chem. Soc., A*, 1969, 2718.
21 W. R. Harris, G. McLendon, and A. E. Martell, *J. Am. Chem. Soc.*, 1976, **98**, 8378.

Received 1*st November* 1982; *Paper* 2/1837