Complexes of Copper(II) Ion with Histamine-containing Dipeptides: Formation Constants and Structure†

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The acid–base properties and copper(II) complexes of glycyl- and sarcosyl-histamine (histamine = imidazole-4-ethanamine) have been studied by pH-metric, spectrophotometric and EPR methods, and compared to those of analogous histidine-containing dipeptides on the one hand and of carcinine (β-alanylhistamine) on the other. At pH 4–9 the predominant species is the 3N-co-ordinated complex $CuLH_{-1}$ (charges omitted) together with minor quantities of CuLH and CuL. The pK for metal ion-promoted deprotonation of the peptidic nitrogen is exceptionally low (pK = 3.20 and 3.66, respectively). The bis complexes CuL_2 and CuL_2H_{-1} also form in the presence of ligand in excess. Around pH 9–11 the monomeric 3N-co-ordinated hydroxo-complex $CuLH_{-1}(OH)$ and a polynuclear 4N-co-ordinated species are in equilibrium. The latter is assumed to be tetrameric $Cu_4L_4H_{-8}$, with the imidazole rings as bridging bidentate units through co-ordination to both N^3 and N^1-pyrrolic nitrogens. At high pH (≈11) a further deprotonation results in the production of the monomeric 3N-co-ordinated hydroxo-complex $CuLH_{-2}(OH)$ with a pendant deprotonated N^1-pyrrolic nitrogen.

The importance of metal-ion interactions with imidazole units in peptides and proteins is now well recognized. Imidazole moieties as side-chain donor groups offer particularly stable metal-binding sites in the physiological pH range. In this respect, imidazole-containing di- and tri-peptides, mostly histidine derivatives such as glycylhistidine (Gly-His),[1–5] histidylglycine (His-Gly),[2–4] glycylhistidylglycine (Gly-His-Gly),[6,7] glycylglycylhistidine (Gly-Gly-His)[8–10] and glycyl-histidyllysine (Gly-His-Lys)[11] have been extensively investigated as suitable models for metalloproteins. Chelates of these multidentate ligands (L) with transition-metal ions, mainly copper(II), have been studied *e.g.* by pH-metric and spectroscopic methods. pH-Metric measurements have shown the presence of monomeric species, increasingly deprotonated through elevation of the pH along the series CuLH, CuL, $CuLH_{-1}$, $CuLH_{-2}$, bis complexes CuL_2 and CuL_2H_{-1}, and oligomeric species, *e.g.* $Cu_2L_2H_{-2}$ or $Cu_4L_4H_{-8}$ The binding sites, especially in multiliganded (bis) species, cannot always be assigned with confidence, due to the large variety of donor atoms: oxygen atoms from carbonyl, carboxylate, co-ordinated water (or hydroxide after hydrolysis); nitrogen atoms from amino, deprotonated amido or N^3-imidazole groups. Important factors are deprotonation of amido and N^1-imidazole nitrogens, the pK values of which are substantially lowered as a result of metal-ion complexation.[12] Copper(II) complexes involving three or four co-ordinated N atoms were shown to be predominant in the physiological pH range. At higher pH the imidazole rings may serve as bridging units doubly co-ordinated to two copper(II) ions in polynuclear species such as $Cu_4L_4H_{-8}$. The formation constants of all the species mentioned above, controlling their predominance or their absence, seem to depend critically on the position of the histidyl residue in the sequence of amino acids, which determines the co-ordination possibilities of the remaining donor groups.

Recently we reported the complex formation of carcinine {β-alanylhistamine, 3-amino-*N*-[2-(imidazol-4-yl)ethyl]propanamide}, a peptide-type derivative of histamine (imidazole-4-ethanamine), of biological interest.[13] This compound was compared to the analogous histidine-containing dipeptide, namely carnosine (β-alanylhistidine). A noteworthy feature in the copper(II)–carcinine system is the formation of a CuL_4H_2 complex at physiological pH with square-planar co-ordination of copper(II) by four monodentate N^3-imidazole nitrogens. At higher pH we found an equilibrium between a 3N monomeric $CuLH_{-1}(OH)$ and a 4N oligomeric species, probably the tetramer $Cu_4L_4H_{-8}$. In the present paper we continue our comparisons between analogous histidine- and histamine-containing dipeptides, using an N-terminal α-amino residue instead of a β-amino residue as in carcinine. We thus report acid–base and spectroscopic properties of copper(II) complexes of glycylhistamine (Gly-Hist) and sarcosylhistamine (Sar-Hist). α-Amino acid residues are expected to introduce important differences in the stability of copper(II) complexes (as compared to *e.g.* carcinine) through the possible formation of five-membered chelated rings (instead of six-membered chelation only, in carcinine).

Experimental

Materials.—Glycyl- (or sarcosyl-)histamine was synthesised in our laboratory from *N-tert*-butoxycarbonyl-glycine or -sarcosine and histamine hydrochloride (Sigma products) according to a procedure to be described.[14] The structure of these compounds was confirmed by ^1H NMR spectroscopy, and their purity checked by NMR, elemental analysis (C, H, N, Cl) and acid–base titration. The $Cu(ClO_4)_2$ (Fluka) stock solution was standardized complexometrically.

Methods.—Formation constants for the general equilibrium (1) (charges omitted) were calculated from potentiometric

$$pM + qL + rH \rightleftharpoons M_pL_qH_r \qquad (1)$$

titration data with the aid of the PSEQUAD computer program.[15] X-Band EPR spectra were recorded at room temperature. The EPR parameters were obtained using the program package described by Szabo-Planka *et al.*[16] Other EPR, UV/VIS and pH-metric experimental procedures were as described previously.[13]

† *Non-SI unit employed:* G = 10^{-4} T.

Table 1 Stability constants (as their logarithms) and derived data for copper(II) complexes of histamine- or histidine-containing dipeptides, $\beta_{pqr} = [Cu_pL_qH_r]/[Cu]^p[L]^q[H]^r$, $I = 0.1$ mol dm^{-3} $NaClO_4$, $T = 298$ K, with estimated errors in parentheses (last two digits)

β_{pqr}	Gly-Hist	Sar-Hist	Carcinine[a]	Gly-His	Gly-His-Gly[b]
001	8.04 (01)	8.31 (01)	9.23	8.22,[c] 8.20[d]	7.99
012	14.82 (01)	15.08 (01)	16.07	14.99,[c] 14.95[d]	14.49
013	—	—	—	17.50,[c] 17.41[d]	17.57
111	11.84 (08)	12.11 (06)	12.78	12.45[c]	—
110	7.19 (10)	7.31 (10)	6.91	9.06,[c] 9.14[d]	9.35
120	14.50 (01)	14.47 (02)	—	15.96,[c] 16.53[d]	15.96
142	—	—	34.10	—	—
11 − 1	3.99 (01)	3.65 (01)	−0.36	4.91,[c] 4.88[d]	5.66
11 − 2	−5.46 (01)	−5.93 (01)	−10.6	−4.84[d]	−3.70
11 − 3	−16.76 (02)	−17.90 (10)	—	—	—
12 − 1	6.90 (01)	6.49 (01)	—	8.02,[c] 8.44[d]	8.32
44 − 8	−14.53 (05)	−15.17 (03)	−33.24	—	−7.20
pK_{CuL}^{CuLH}	4.65	4.80	5.87	3.39[c]	—
$pK_{CuLH_{-1}(NH)}^{CuLH}$	3.20	3.66	7.27	4.15,[c] 4.26[d]	3.69
$pK_{CuLH_{-1}(OH)}^{CuLH}$	9.45	9.58	10.24	9.72[d]	9.36
$pK_{CuLH_{-2}(OH)}^{CuLH_{-1}(OH)}$	11.30	11.97	—	—	—
$pK_{CuL_2H_{-1}(NH_3)}^{CuL_2H_2}$	7.60	7.98	—	7.94,[c] 8.09[d]	7.64
$\log K_{CuL_2}^{CuLH_{-1}(LH)}$	2.47	2.51	2.48[e]	2.83,[c] 3.45[d]	2.31

[a] From ref. 13. [b] From ref. 6. [c] From ref. 4. [d] From ref. 3. [e] β_{140} for copper(II)–*N*-*tert*-butoxycarbonylcarcinine from ref. 13.

Results

The pH-metrically determined protonation constants of the free ligands studied are listed in Table 1, together with those of carcinine, glycylhistidine, and glycylhistidylglycine for comparison. Formation constants β_{011} and β_{012} can be approximately assigned to protonation of the amino groups and N^3-imidazole nitrogen, respectively. However, as the two deprotonations overlap significantly, alternative microprotonation (N^3 of imidazole is deprotonated while the amino group remains protonated) can also occur to a minor extent. The β_{011} value of Sar-Hist is slightly higher than that of Gly-Hist, because of the I^+ inductive effect of the *N*-methyl substituent. The decreased basicity of the terminal amino group in both compounds, compared to that of carcinine (see Table 1), is attributed to the closer electronegative carbonyl group in an α- than in a β-amino acid residue.

Formation constants of the copper(II) complexes were obtained from pH-metric titration curves for each ligand drawn at a given metal ion to ligand ratio *R*. For an equimolar solution ($R = 1:1$) the pH-metric plots of either ligand (Gly- or Sar-Hist) show an inflection point between pH 6 and 8, after the addition of 3 base equivalents per metal ion. In line with earlier findings for this type of ligand, this observation strongly suggests the predominant formation of $CuLH_{-1}$ species (charges omitted) in this pH range, through the co-ordination of N^3-imidazole, amino and peptide nitrogens. Further deprotonation processes take place on increasing the pH from 8–9 to *ca.* 11.2 with the consumption of 1.4–1.7 additional base equivalents. Two processes are generally proposed to account for this additional base consumption: hydrolysis of the complex and deprotonation of the N^1-pyrrolic nitrogen of imidazole. Both are involved in the present case since more than 1 base equivalent is required to reach pH 11. The titration plots for molar ratios *R* smaller than unity, *e.g.* for $R = 1:2$, differ from the curves which can be computed on the basis of a simple excess of ligand in the presence of the species found with $R = 1:1$. This shows the additional formation of multiliganded and/or polynuclear species, as in the case of carcinine.[13]

The nature of the complexes in solution was established not only on the basis of the base consumption determined pH-metrically, but also on spectroscopic observations. Although essentially qualitative in nature, spectroscopic data are invaluable to assess the structure of copper(II) complexes. Three criteria were mainly used as described in a previous publication:[13] (*a*) the UV/VIS position of the d–d band (λ_{max}^{d-d}) characteristic of equatorial nitrogen or oxygen co-ordination to copper(II); (*b*) EPR parameters, g_0 values and hyperfine constants A_0, the variations of which are indicators for changing species; and (*c*) EPR superhyperfine patterns at high field when the fine structure is sufficiently resolved. The number and intensity of superhyperfine lines gives the number of ^{14}N nuclear spins coupled to the electronic spin from the metal ion, *i.e.* the number of nitrogen atoms equatorially co-ordinated to copper(II): a septet, or a nonet, is thus characteristic for 3N- or 4N-co-ordinated species, respectively. It is of interest that, for all complexes studied by EPR spectroscopy, good fitting can be achieved by assuming equivalent nitrogen donors (although there are three different types of nitrogen in the ligands). Plots of λ_{max}^{d-d} as a function of the analytical concentration of copper(II) for a series of *R* values are especially useful to make the distinction between polynuclear and multiliganded complexes, as explained previously.[13]

Data from all titrations for each system were treated simultaneously in refining the sets of constants given in Table 1. However, the number of species actually present within various pH ranges and at given ligand and metal-ion concentrations is much more restricted. Preliminary estimates were thus carried out in a first step using reduced lists of parameters. Distribution curves of species computed on the basis of the formation constants thus determined are shown in Figs. 1 and 2. Spectroscopic parameters extracted for each individual species from experimental data (maximum UV/VIS absorption wavelength λ_{max}^{d-d} and absorption coefficient ε; EPR parameters g_0 and A_0) are given in Table 2.

Discussion

Complexes in the Range pH 4–8.—Starting from an acidic, equimolar solution, first the CuLH then the CuL complexes form for both ligands. These are only minor species, thus they could not be identified spectroscopically. However, as in the free ligand the deprotonations of the amino and imidazole group overlap, it can be assumed that the first metal-promoted deprotonation can also occur at either nitrogen. The slight increase in $\log K_1$ ($Cu + HL \rightleftharpoons CuLH$, $\log K_1 = \log \beta_{111} - \log \beta_{011}$) of Gly- and Sar-Hist compared to carcinine (due to the formation of a five- instead of a six-membered chelate, respectively, between the terminal amino and carbonyl group, see Table 1) also supports the (minor) participation of the terminal amino group in the first co-ordination step, however it contributes more substantially at the second metal-promoted deprotonation ($CuLH \rightleftharpoons CuL + H^+$, $pK_{CuL}^{CuLH} = \log \beta_{111} -$

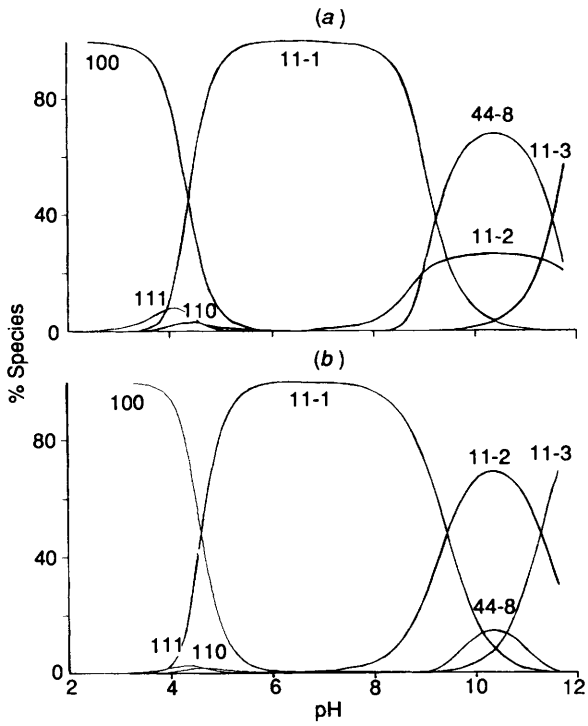


Fig. 1 Concentration distribution of complexes found in the Cu^{II}–Gly-Hist system as a function of pH, at constant metal ion to ligand ratio $Cu^{II}:L = 1:1$ and $[Cu^{II}] = 1.2 \times 10^{-2}$ (*a*) and 2×10^{-3} mol dm^{-3} (*b*)

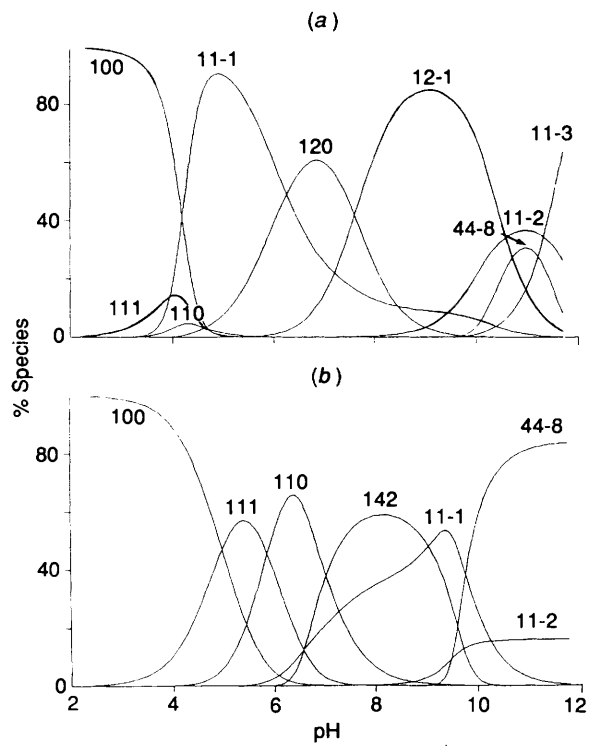


Fig. 2 Concentration distribution of copper(II) complexes with glycylhistamine (*a*) compared to the analogous copper(II)–carcinine (*b*) system,[13] $Cu^{II}:L = 1:4$ and $[Cu^{II}] = 6 \times 10^{-3}$ mol dm^{-3}

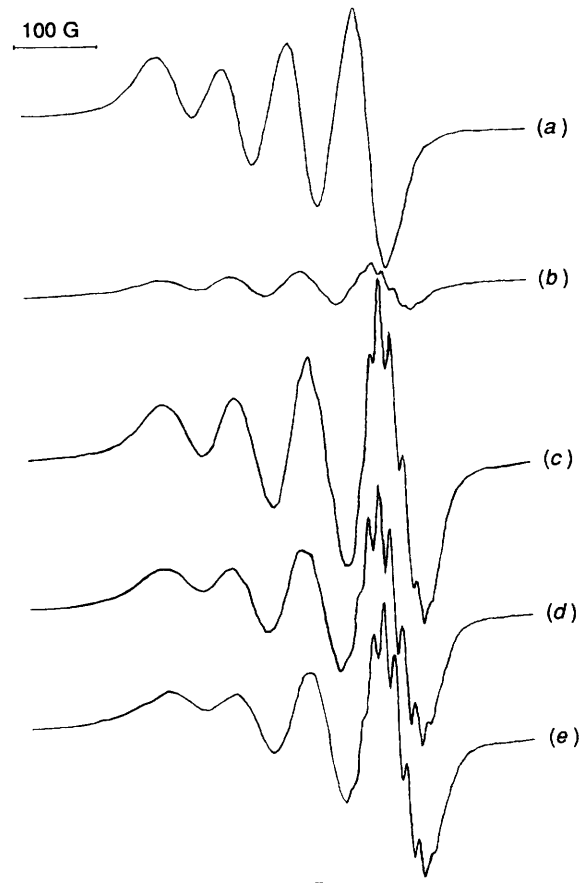


Fig. 3 The EPR spectra of Cu^{II}–Gly-Hist solutions at room temperature, with $Cu^{II}:L = 1:1$, $[Cu^{II}] = 6 \times 10^{-3}$ mol dm^{-3} and at pH 7.2 (*a*), 11.0 (*b*) and > 12 (*c*); $Cu^{II}:L = 1:20$, $[Cu^{II}] = 4 \times 10^{-3}$ mol dm^{-3} at pH 7.2 (*d*) and 9.3 (*e*). Predominant complexes in each case are respectively $CuLH_{-1}$, $CuLH_{-1}(OH)$ and $Cu_4L_4H_{-8}$ (undetected), $CuLH_{-2}(OH)$, CuL_2, CuL_2H_{-1}. Note that for all spectra the same amplifications have been employed

$\log \beta_{110}$) bringing about a larger decrease of pK_{CuL}^{CuLH} from 5.87 (for carcinine) to 4.65 and 4.8 (for Gly- and Sar-Hist). In the range pH 4–8 the predominant species is $CuLH_{-1}$. This complex involves a 3N equatorial co-ordination of N^3-imidazole, amino and amido nitrogens, as is clearly shown by the d–d absorption maximum $\lambda_{max}^{d\text{-}d} = 601$ nm of the Gly-His complex, which is in good agreement both with experimental values observed for similar complexes involving closely related ligands, such as Gly-His (600 nm)[4] and Gly-His-Gly (605 nm),[6] and with the theoretically calculated one (593 nm) using increments from the literature.[17] The EPR superhyperfine structure is too poorly resolved [Fig. 3(*a*)] to provide any information on the number of co-ordinated nitrogens. However the overall EPR parameters (g_0 and A_0, Table 2) are nearly identical to those determined for the analogous Cu^{II}–Gly-His system,[5] which brings additional support to the 3N-co-ordinated structure of complex $CuLH_{-1}$. The p*K* for the metal-ion-promoted deprotonation of the peptide bond is indeed exceptionally low (Table 1): pK_{NH} = 3.20 and 3.66 (Gly- and Sar-Hist, respectively), against 7.27 in carcinine. This dramatic fall can be assigned to an easier formation of (5,6) chelates in the present case as opposed to (6,6) chelates with carcinine. This high acidity of the peptide bond accounts for the shift in species distribution curves towards the acidic end of the graph, and for the 100% predominance of $CuLH_{-1}$ from pH 6 to 8, when the metal ion to ligand ratio *R* is maintained at 1:1.

It should be noted that the presence of an additional carboxylate binding site in the analogous Gly-His ligand has a small effect in the opposite direction on the p*K* of the amide hydrogen (pK_{NH} = 4.15) compared with pK_{NH} = 3.20 for Gly-Hist. The decrease in acidity of Gly-His is presumably due to the negative charge on the carboxylate which hinders removal of the peptidic proton. This interpretation is confirmed by observing that the pK_{NH} is also very low when the terminal acidic end of histidine is removed by forming the tripeptide X-His-Y, *e.g.* Gly-His-Gly[6,7] ($pK_{NH} \approx 3.6$) or Gly-His-Lys[11] (pK_{NH} = 2.88).

Table 2 The UV/VIS data (λ_{max}^{d-d} and ε for d–d band) and EPR parameters (g_0 and A_0) for copper(II) complexes with Gly- and Sar-Hist *

	Gly-Hist				Sar-Hist			
Complex	λ_{max}^{d-d}/nm	ε/dm^3 mol^{-1} cm^{-1}	g_0	A_0/G	λ_{max}^{d-d}/nm	ε/dm^3 mol^{-1} cm^{-1}	g_0	A_0/G
$CuLH_{-1}$	601	62	2.119	74.6	604	72	2.121	75.0
$CuLH_{-2}$	574	83	2.109	78.3	569	87	2.113	77.7
$CuLH_{-3}$	556	79	2.106	83.7	559	77	2.107	83.0
CuL_2	563	68	2.110	83.0	568	76	2.109	82.0
CuL_2H_{-1}	568	70	2.103	82.0	567	76	2.109	82.0
$Cu_4L_4H_{-8}$	550	358	—	—	552	344	—	—

* Estimated errors: g, ±0.001; A, ±0.3; λ_{max}^{d-d}, ±3; ε, ±3.

In the presence of higher concentrations of copper(II) ion the question arises as to the possible formation of dimeric complexes, as shown in the case of the Cu^{II}–His-Gly or copper(II)–carnosine systems.[4] It should however be observed that, in the examples cited above, each ligand has four binding sites (CO_2^-, N^-, NH_2 and N^3), one of which [N^3] plays the role of bridging unit. This is not the case with histamine-containing dipeptides, which may thus be expected to yield monomeric or multiganded complexes only at this pH. This was confirmed by our UV/VIS spectroscopic measurements at different total copper(II) concentrations and given metal ion to ligand ratios R.

In the presence of an excess of ligand, *i.e.* for metal ion to ligand ratios smaller than 1 : 1, bis(ligand) complexes CuL_2 and CuL_2H_{-1} also form in the range pH 6–10 [Fig. 2(*b*)]. Both the λ_{max}^{d-d} value of these species (Table 2) and the well resolved nonet superhyperfine pattern of the high-field EPR lines [Fig. 3(*d*) and 3(*e*)] fully support the co-ordination of copper(II) to four nitrogens in the equatorial plane. The UV/VIS and EPR parameters are quite similar for both species. Moreover the pK for the deprotonation of ML_2 to give ML_2H_{-1} (pK = 7.60 for L = Gly-Hist) is fairly close to that observed (pK = 8.04) for the free ligand (HL) for the deprotonation of the ammonio group. This suggests the existence of a free NH_3^+ group in the complex CuL_2, which remains unco-ordinated after deprotonation to NH_2 to give the complex CuL_2H_{-1}. In this respect the complexes CuL_2 and CuL_2H_{-1} should be denoted as $Cu(LH_{-1})(HL)$ and $Cu(LH_{-1})L$. Their formation would result from the reaction of $CuLH_{-1}$ (which is predominant in this pH range, see above) with the excess of ligand HL according to equation (2) with an equilibrium constant computed as in (3).

$$CuLH_{-1} + HL \rightleftharpoons Cu(LH_{-1})(HL) \quad (2)$$

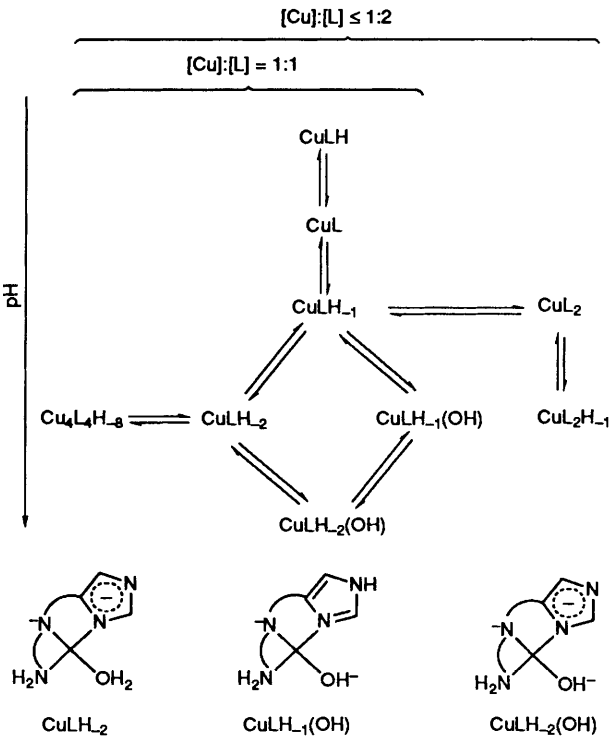
$$\log K_{CuL_2}^{CuLH_{-1}} = \log \beta_{120} - \log \beta_{11-1} - \log \beta_{011} \quad (3)$$

An interesting piece of evidence supporting this view is to consider the equilibrium constants for the monodentate attachment of protected *N-tert*-butoxycarbonylcarcinine (A) to copper(II) along the series of consecutive reactions (4) where

$$CuA_n + A \overset{K_n}{\rightleftharpoons} CuA_{n+1} \quad (4)$$

n = 0–3 and log K_n = 3.85, 3.40, 2.68 and 2.48, respectively.[13] More especially, the attachment of ligand A to the fourth free site about copper(II) is characterized by a value of log K_3 = 2.48 which is nearly identical to log $K_{CuL_2}^{CuLH_{-1}}$ (2.47 in the Cu^{II}–Gly-Hist system). This strongly suggests the monodentate attachment of an N^3-imidazole nitrogen to the fourth co-ordination site of copper(II) as a common step in the formation of either CuA_4 or $Cu(LH_{-1})(HL)$.

Decreasing the metal ion to ligand ratio well below 1 : 2 does not bring significant changes in the UV or EPR spectra. This rules out the presence of multiliganded complexes in which more than one ligand molecule is co-ordinated in a monodentate manner to copper(II) through N^3-imidazole nitrogens, as in the above-mentioned complexes CuA_n of *N-tert*-butoxycarbonylcarcinine, or in the complex CuL_4H_2 of carcinine. In this respect carcinine and its derivative appear to be exceptional, which can presumably be ascribed to the presence of a β-alanyl residue which makes the (6,6) chelated $CuLH_{-1}$ species less stable, thus promoting its conversion into multiliganded complexes with monodentate co-ordination.



Scheme 1 Species in the system Cu^{II}–Gly- (or Sar)-Hist at different stages of deprotonation (increasing pH along the vertical axis) with the proposed structure of some species in the equatorial plane of copper(II)

Complexes in the Range pH 8–11.—Starting from the predominant species $CuLH_{-1}$ at pH ~ 8, further deprotonation processes take place forming the monomeric $CuLH_{-1}(OH)$, $CuLH_{-2}(OH)$ and a probably tetrameric ($Cu_4L_4H_{-8}$) species. In the monomeric complexes the first base consumption [$CuLH_{-1} \rightleftharpoons CuLH_{-1}(OH) + H^+$, $pK_{CuLH_{-1}(OH)}^{CuLH_{-1}} = 9.45$ for Gly-Hist] can be attributed to water deprotonation at the fourth co-ordination position, and the second one [$CuLH^{-1}(OH) \rightleftharpoons CuLH_{-2}(OH^-) + H^+$, $pK_{CuLH_{-2}(OH)}^{CuLH_{-1}(OH)} = 11.3$ for Gly-Hist] to deprotonation of N^1-pyrrolic nitrogen of imidazole. Since the overlapping of the last two deprotonations is not significant, the constants can be regarded as macroconstants. However there is a possibility, although in a few % only, of the formation of an alternative microstructure $CuLH_{-2}$, in which N^1 is deprotonated instead of the equatorially co-ordinated water (see Scheme 1). This deprotonation pathway

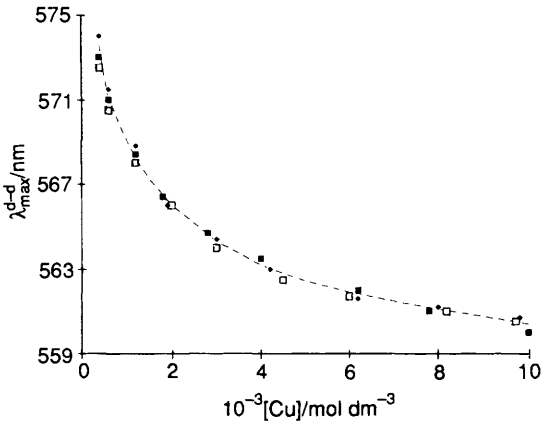


Fig. 4 Positions of the d–d band maximum in the Cu^{II}–Gly-Hist system as a function of the total copper(II) concentration at pH 11.0 and Cu^{II}:L = 1:1 (◆), 1.2 (□) and 1:4 (■)

should be always subordinate and this is, in fact, observed in diluted solutions. At higher concentrations, however, an oligomerization process changes fundamentally the importance of the latter microdeprotonation pathway, due to the favoured substitution of a water molecule in the fourth position of the $CuLH_{-2}$ species by the N^1-pyrrolic nitrogen. Both nitrogens of one imidazole ring cannot however be co-ordinated to the same metal ion on steric grounds. This is only possible in polymeric species in which each imidazole ring bridges two copper(II) ions. This involves in turn using concentrated copper(II) solutions so as to shift the usual microdeprotonation equilibrium towards the formation of the polynuclear species $(CuLH_{-2})_n$ (*via* $CuLH_{-2}$, see Scheme 1), equation (5). A tetrameric species

$$n(CuLH_{-2}) \rightleftharpoons (CuLH_{-2})_n \quad (5)$$

$Cu_4L_4H_{-4}$, consisting of a nearly planar closed loop formed with four monomeric units $CuLH_{-2}$, has been suggested in earlier work on the Cu^{II}–Gly–His[1,4] and the Cu^{II}–Gly-His-Gly systems,[6] and reconsidered in our previous investigations on the copper(II)–carcinine system.[13] A cyclic tetrameric structure has been effectively described recently by X-ray crystallography for the system Au^{III}–Gly-His.[18] The complex $[\{Au^{III}(Gly\text{-}L\text{-}His)\}_4]\cdot 10H_2O$ is quite similar to the copper(II) complexes investigated presently, since the co-ordination geometry of Au^{III} is also square planar with four N donors. The set of four gold ions, linked to each other by bidentate imidazole rings, is arranged at the vertices of a distorted tetrahedron of symmetry C_2. These results strongly support the possibility of a similar structure in the case of the analogous copper(II) complexes.

The simultaneous presence of two species, $CuLH_{-1}(OH)$ and $Cu_4L_4H_{-8}$, is revealed in Fig. 4 by plots of λ^{d-d}_{max} as a function of copper(II) concentration at a constant pH of 11.0 and different metal ion to ligand ratios (from 1:1 to 1:4). At lower copper(II) concentrations the hydroxo complex is predominant. The corresponding d–d band is shifted by 27 nm to the blue compared to $CuLH_{-1}$, in line with a higher ligand-field strength of OH^- [in $CuLH_{-1}(OH)$] compared to H_2O (in $CuLH_{-1}$). The septet superhyperfine pattern observed in the EPR spectrum [Fig. 3(*b*)] also confirms the 3N co-ordination in these complexes. On increasing the copper(II) concentration (Fig. 4) there is an additional blue shift of the d–d band by about 24 nm, showing the progressive formation of a new species at the expense of the hydroxo complex. The magnitude of the blue shift strongly suggests 4N co-ordination in this new species. The fact that this shift is not dependent upon the metal ion to ligand ratio rules out the possibility of a multiliganded species, and is in favour of polymeric 4N-co-ordinated species, such as the tetramer described above. Simultaneously, the EPR signal intensity is reduced by *ca.* 80% [Fig. 3(*b*)], which suggests a possible antiferromagnetic interaction among copper(II) atoms, closely linked to each other within a polymeric sequence involving an even number of monomers. The tetrameric complex $Cu_4L_4H_{-8}$ seems to fit best the above conditions (evenness of the number of monomers and interatomic distances), and this is presumably the first spectroscopic evidence directly supporting this structure in solution. On the basis of EPR signal intensity, there remains *ca.* 20% of the monomeric complex $CuLH_{-1}(OH)$ in solution, in good agreement with the distribution curves (Fig. 1) deduced from pH-metric measurements.

Further addition of base is required to raise the pH above 11. The pH elevation cannot be reduced to the sole existence of hydroxide ion in excess, on the basis of first the observed titration curves, secondly of the intensity of the EPR signal which progressively recovers its full amplitude [Fig. 3(*c*)], and thirdly of the well resolved structure of the EPR spectrum indicating a 3N co-ordinated complex. All these facts are in favour of a monomeric species $CuLH_{-2}(OH)$, as a result of deprotonation of both the equatorially co-ordinated water molecule and N^1-pyrrolic nitrogen. The same oligomerization process for this species as that described above for $CuLH_{-2}$ is probably prevented by the much greater basicity and nucleophilicity of OH^- compared to H_2O. In consequence of the change in electronic structure of the imidazole ring, caused by copper(II) co-ordination at the N^3 nitrogen, the pK value for this deprotonation decreases by about three log units as compared to the free ligand and the values determined agree well with that found for prolylhistidine, a related compound.[19]

Conclusion

The copper(II) complexation of the histamine-containing dipeptides studied has specific features sharply contrasting with those previously found for carcinine. In acidic media the monomeric complexes CuLH and CuL are only minor species. The predominant species at pH between 5 and 8 is the monomeric 3N-co-ordinated complex $CuLH_{-1}$. The exceptionally low pK values for metal deprotonation of amide nitrogen result from a very stable (5,6)-chelate formation which is reminiscent of that observed with Gly-His, a histidine-containing dipeptide, where this pK is however somewhat higher, presumably because of the negative charge on the neighbouring carboxylate end. In the presence of ligand in excess, bis complexes $[Cu(LH_{-1})HL$ and $Cu(LH_{-1})L]$ also form, with the N^3 nitrogen as the only binding site for the second ligand. The series of monomeric affiliated species $CuLH_{-1}$, $CuLH_{-1}(OH)$ and $CuLH_{-2}(OH)$, although having a common 3N co-ordination to copper(II), is characterized by a gradual shift in EPR parameters (g_0 decreases, A_0 increases) and of the d–d band to the blue, indicating a progressive change in the ligand-field strengths along the series. In basic solution, a tetrameric species $Cu_4L_4H_{-8}$ also forms, with the co-ordination of both N^3 and N^1 nitrogens of the imidazole bridging units. Finally, the formation constants measured for the monomeric copper(II)–Sar-Hist species are slightly smaller than those found for the Gly-Hist complexes because of the weak steric hindrance from the *N*-methyl substituent. All these facts illustrate the wealth of the solution chemistry of peptide complexes and the influence of structural details in these ligand molecules over the nature and concentration of the species. They also contribute to delineating the specific features brought about by the presence of a β-amino acid residue in a peptidic sequence.

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