Thermodynamic and Spectrophotometric Study of Copper(II) and Cadmium(II) Homo- and Hetero-nuclear Complexes with L-Histidylglycine in an Aqueous Medium

Pier G. Daniele,* Orfeo Zerbinati, Roberto Aruga, and Giorgio Ostacoli
Department of Analytical Chemistry, University of Turin, Via Giuria 5, 10125 Turin, Italy

Homo- and hetero-nuclear complexes of copper(II) and cadmium(II) with L-histidylglycine have been investigated by potentiometry, visible spectrophotometry, and calorimetry in aqueous medium, at T = 25 °C and I = 0.1 mol dm^{-3} (KNO_3). Gibbs functions obtained by potentiometry, combined with calorimetric enthalpies, gave the entropies of formation of the complex species. Experimental data have been obtained for the following species (L^- = histidylglycinate anion): HL, H_2L^+, H_3L^{2+}, $[CuL]^+$, $[CuL_2]$, $[CuLH]^{2+}$, $[CuL_2H]^+$, $[Cu_2LH_{-1}]^{2+}$, $[Cu_2L_2H_{-2}]$, $[CdL]^+$, $[CdL_2]$, $[CdLH]^{2+}$, $[CuCdLH_{-1}]^{2+}$, $[CuCdLH_{-2}]^+$. In general, the structures predicted on the basis of the spectrophotometric and thermodynamic results are in good agreement.

Complexes of peptides with metal ions have aroused increasing interest in recent years. The first studies were on metal complexes of polyglycines, the behaviour of the peptide group and, in particular, its acid dissociation being the most interesting problem at that stage.[1]

More recently, most papers in this field have dealt with the complexes of histidine-containing peptides. This interest is mainly for biological reasons. It is well known, in fact, that the imidazole group of histidine plays a fundamental role in several metal–protein and metal–enzyme reactions of living organisms.[2] Copper(II), for instance, is transported in blood as the complex with human seroalbumin.[3,4] In this complex-forming process the histidine group plays a leading role. Thus the simplest histidine-containing peptides may be used as models for more complex biological agents.

Potentiometry and u.v.–visible spectrophotometry are the most widely used methods in the investigation of metal–histidylpeptide complexes. N.m.r. and e.s.r. spectroscopy have been used to a lesser extent, and there have been only a few calorimetric investigations.[5–7]

For metal complexes of L-histidylglycine (hisgly, HL), only poor agreement of previous conclusions about the species formed and the deprotonating sites of the dipeptide in dimeric species has been obtained and, consequently, there has been difficulty in assigning a definite structure to the corresponding complex species with copper(II).[6,8–11] This is significant, as certain bi- and tri-dentate complexes of copper(II), bound by imidazole bridges (with or without the deprotonation of the pyrrole nitrogen), were shown to be important as models for the active site of bovine erythrocyte superoxide dismutase.[12]

While, for example, a contemporaneous participation of the amine and the imidazole nitrogen in hisgly complexes of the type $[CuL]^+$ and $[CuL_2]$ is generally admitted, a glycine-type structure (with the participation of the amine nitrogen and the peptide oxygen only) is shown by e.s.r. spectroscopy for the complexes of histidylalanine, the structure of which is quite similar to that of hisgly.[13]

The presence of heteronuclear (or mixed-metal) complex species has aroused interest in the study of amino acid and peptide complexes. It has been shown that at pH 6—9, in the presence of several metal ions, these ligands exist mainly in the form of heteronuclear complexes. Such species are of importance in the study of biofluids, particularly when hyperaccumulated metal ions are present for physiological or pathological reasons.[14] Mutual influences between metal ions may be of antagonist or of synergistic nature;[15] however the causes of these influences are not yet satisfactorily understood.

Higher stabilities of hetero- compared with corresponding homo-nuclear species have been attributed to different 'hard' and 'soft' characters of binding sites and of metal ions;[16] however this factor may be of minor importance[14,17] while possible reciprocal influences of the co-ordinating sites of the ligand may be more crucial.

In view of the preceding observations, and also of the lack of enthalpy and entropy data for metal–L-histidylglycine species, we have investigated homo- and hetero-nuclear complexes of copper(II) and cadmium(II) with hisgly in an aqueous medium by potentiometry, calorimetry, and visible spectrophotometry.

Experimental

Chemicals.—L-Histidylglycine (HL) was a Sigma product. Its purity was checked by ion chromatography and by alkalimetric titration. Metal nitrate stock solutions were prepared and standardized as previously reported.[18] All the standard solutions were prepared by using twice-distilled water. Ionic strength was adjusted to 0.1 mol dm^{-3} by addition of KNO_3.

E.M.F. Measurements.—Potentiometric measurements were performed with a Metrohm E600 potentiometer equipped with glass and calomel electrodes supplied by the same firm. The calibration of the glass electrode, in $-\log[H^+]$ units (pH), both in acidic and alkaline regions was made by titrating nitric acid (4—6 mmol dm^{-3}) with standard, carbonate-free KOH. The ionic composition and the ionic strength of the calibrating solution were the same as that of the solutions being examined. The experimental conditions of the alkalimetric titrations are shown in Table 1.

Spectrophotometric Measurements.—The visible spectrophotometric determinations were carried out with a Spectracomp 601 Carlo Erba spectrophotometer from 400 to 800 nm. Spectra were recorded on solutions for which both total reagent concentrations and pH were known. The experimental conditions are the same as those reported in Table 1 for potentiometric measurements.

Calorimetric Measurements.—The calorimetric experiments were carried out at 25.00 ± 0.02 °C and I = 0.1 mol dm^{-3} (KNO_3) using a LKB 2107 microcalorimeter (batch calorimeter with heat-flux measuring system). The accuracy and the

reproducibility of the system were tested by measuring the heat of ionization of water at 25 °C and $I = 0.1$ mol dm^{-3} (KNO_3); a value of -56.0 kJ mol^{-1} ($3\sigma = 0.3$) was found. The measurements were performed in a room kept at a temperature constant within ± 0.5 °C. For every measurement 1—10 mg of peptide were accurately weighed, using a Sartorius microbalance (minimum reading 10^{-6} g). The calorimetric measurements were carried out by adding to 4-cm^3 solutions of desired composition known amounts of nitric acid, potassium hydroxide, or metal nitrate stock solution, in order to displace the complexation equilibria. The dilution and friction heats were taken into account by placing the titrant solution and 0.1 mol dm^{-3} KNO_3 in the two reference cells.

To investigate the present binary and ternary systems, 166 independent measurements have been performed. The experimental heats ranged between 0.04 and 1.20 J. Some experimental details are reported in Table 2.

Data Analysis and Calculations.—Up to pH 9—9.5, in the absence of metal ions, three hydrogen ions per molecule of hisgly can dissociate, from the carboxylate, imidazole, and amino groups. So we considered the fully protonated form of this ligand as $[H_3L]^{2+}$. In the presence of metal ions further deprotonation may occur, from peptide and pyrrole groups.

The stability constants are expressed by the general equation given below. The stability constants of the binary complexes

$$\beta_{pp'qr} = [Cu_pCd_{p'}L_qH_r]/[Cu]^p[Cd]^{p'}[L]^q[H]^r$$

(Cu^{II}–L, Cd^{II}–L) were calculated by means of SUPERQUAD,[19] which minimizes the error-square sum on e.m.f. values. The existence of ternary mixed-metal complexes was then inferred from the comparison between the experimental alkalimetric titration curves (obtained in the presence of Cu^{II}, Cd^{II} and the ligand) and those calculated by taking into account only all the binary species. At pH >5 there was a significant positive difference, for the same pH, between experimental and calculated values of added alkali concentration, thus indicating that homonuclear species were likely to exist. The type of heterobinuclear complexes and their stability constant values were successively determined by SUPERQUAD.

Analysis of spectrophotometric data was made by means of the MOLEX program,[20] which calculates the values of molar absorptivity coefficients (ε_λ) of the complex species, from experimental spectra and known values of stability constants. The refinement of calorimetric data was made by means of the ES5CM[21] and KK83[22] programs. In all calculations the hydrolysis of Cu^{II}[23] and of Cd^{II}[24] was taken into account.

Table 1. Analytical concentrations (c/mmol dm^{-3}) for potentiometric titrations of L-histidylglycine, Cu^{II}, and Cd^{II} at 25 °C

c_{Cu}	c_L	c_{Cd}	c_L	c_{Cu}	c_{Cd}	c_L
2.5	2.5	5.0	5.0	3.0	12	6.0
3.5	3.5	6.0	6.0	3.0	15	3.0
5.0	5.0	2.0	6.0	3.5	15	3.6
1.5	3.0	2.5	5.0	2.5	12	5.2
2.0	4.0	3.0	4.5	4.0	12	4.0
2.5	5.0	15	3.0			
6.0	2.5					
8.0	3.0					
9.0	3.5					

Results

Copper–L-Histidylglycine System.—The refinement of potentiometric readings derived from alkalimetric titrations for which $c_{Cu} \leqslant c_L$ by SUPERQUAD has shown that experimental

Table 2. Experimental details of calorimetric measurements at 25 °C (analytical concentrations, c in mmol dm^{-3}, initial volume 4 cm^3)

c_L	$c_H - c_{OH}$		Added reagent	Number of measurements
(*a*) L-Histidylglycinate protonation				
4		4	0.1 cm^3 KOH (0.1 mol dm^{-3})	10
1.2		0.6	0.1 cm^3 HNO_3 (0.03 mol dm^{-3})	45
2.5		6	0.2 cm^3 HNO_3 (0.5 mol dm^{-3})	5
(*b*) Cadmium–histidylglycine complexes				
—	2.8	2	0.66 cm^3 $Cd(NO_3)_2$ (0.1 mol dm^{-3})	5
—	14	24	0.13 cm^3 $Cd(NO_3)_2$ (0.1 mol dm^{-3})	10
—	14	10	0.13 cm^3 $Cd(NO_3)_2$ (0.1 mol dm^{-3})	10
3.2	16	12	0.43 cm^3 HNO_3 (0.1 mol dm^{-3})	5
1.9	10	6.7	0.96 cm^3 HNO_3 (0.1 mol dm^{-3})	15
(*c*) Copper–histidylglycine complexes				
—	6	5.5	0.2 cm^3 $Cu(NO_3)_2$ (0.01 mol dm^{-3})	5
1	7.8	7.5	0.4 cm^3 HNO_3 (0.01 mol dm^{-3})	12
—	1	1.1	0.16 cm^3 $Cu(NO_3)_2$ (0.1 mol dm^{-3})	6
5	5	−4.4	0.3 cm^3 HNO_3 (0.01 mol dm^{-3})	7
1.6	3.2	−0.3	0.1 cm^3 HNO_3 (0.1 mol dm^{-3})	7
4	4	−1.7	60 μmol HNO_3 + 60 μmol L in 0.1 cm^3 KNO_3 (0.1 mol dm^{-3})	5
—	4.4	4.4	0.9 cm^3 $Cu(NO_3)_2$ (0.2 mol dm^{-3})	6
(*d*) Copper–cadmium–histidylglycine complexes				
3	30	2.9	−2.8	0.12 cm^3 HNO_3 (0.1 mol dm^{-3})	7
3	30	2.8	−3.5	0.43 cm^3 HNO_3 (0.01 mol dm^{-3})	6

Table 3. Thermodynamic parameters for $[Cu_pCd_pL_qH_r]$ complexes at 25 °C and I = 0.1 mol dm^{-3} in aqueous solution *

Reaction	log K	$-\Delta G^{\circ}$	$-\Delta H^{\circ}$	ΔS°
(1) $L^- + H^+ \longrightarrow HL$	7.63(1)	43.5(1)	41.4(4)	7(2)
(2) $HL + H^+ \longrightarrow H_2L^+$	5.84(1)	33.3(1)	31.8(4)	5(2)
(3) $H_2L^+ + H^+ \longrightarrow H_3L^{2+}$	2.62(2)	14.9(1)	−1(3)	53(10)
(4) $Cu^{2+} + L^- \longrightarrow [CuL]^+$	8.84(2)	50.4(1)	45.8(6)	15.5(20)
(5) $[CuL]^+ + L^- \longrightarrow [CuL_2]$	6.49(3)	36.8(2)	42(1)	−17(4)
(6) $Cu^{2+} + L^- + H^+ \longrightarrow [CuLH]^{2+}$	12.10(5)	69.0(3)	55(1)	47(4)
(7) $Cu^{2+} + 2L^- + H^+ \longrightarrow [CuL_2H]^+$	20.06(5)	114.3(3)	97.5(20)	56(8)
(8) $2Cu^{2+} + L^- \longrightarrow [Cu_2LH_{-1}]^{2+} + H^+$	5.24(6)	29.9(3)	19(2)	36(8)
(9) $2Cu^{2+} + 2L^- \longrightarrow [Cu_2L_2H_{-2}] + 2H^+$	8.22(4)	46.8(2)	46(2)	3(4)
(10) $Cd^{2+} + L^- \longrightarrow [CdL]^+$	4.55(2)	25.9(1)	22.2(8)	12(4)
(11) $[CdL]^+ + L^- \longrightarrow [CdL_2]$	3.35(5)	19.1(3)	17.5(12)	5(3)
(12) $Cd^{2+} + L^- + H^+ \longrightarrow [CdLH]^{2+}$	9.77(4)	55.7(2)	53(2)	9(6)
(13) $Cu^{2+} + Cd^{2+} + L^- \longrightarrow [CuCdLH_{-1}]^{2+} + H^+$	4.30(6)	24.5(3)	15(2)	32(6)
(14) $Cu^{2+} + Cd^{2+} + L^- \longrightarrow [CuCdLH_{-2}]^+ + 2H^+$	−3.65(6)	−20.8(3)	−15(3)	−19(6)

* ΔG° and ΔH° in kJ mol^{-1}; ΔS° in J K^{-1} mol^{-1}. Values in parentheses are 3σ.

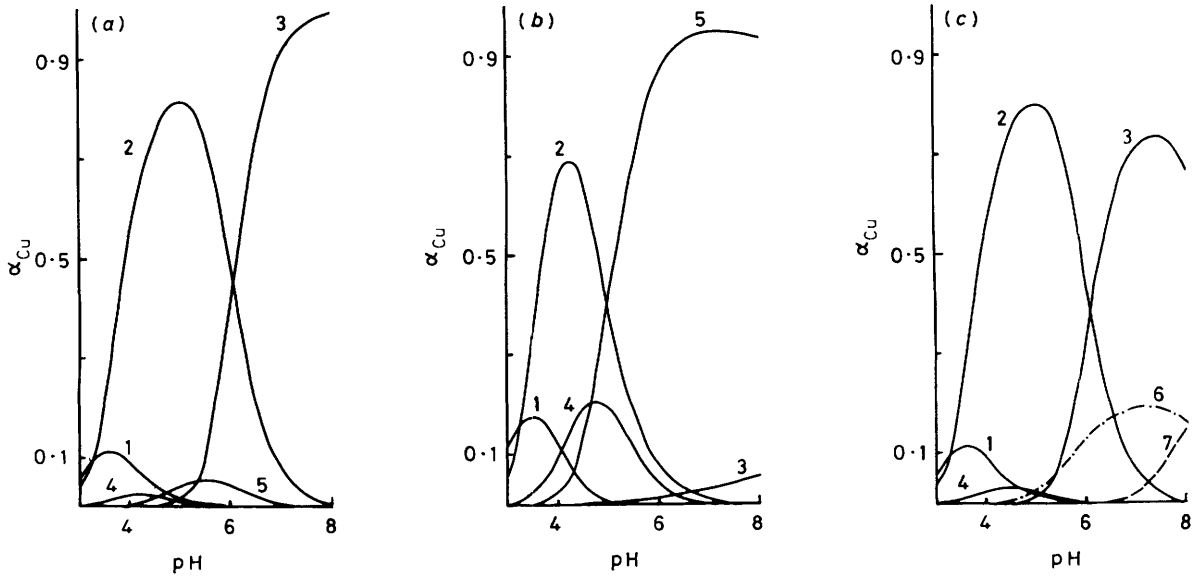


Figure 1. Distribution diagrams (fraction of total Cu, α_{Cu} *vs.* pH): (*a*) c_{Cu} = 2.50, c_L = 2.50 mmol dm^{-3}; (*b*) c_{Cu} = 2.50, c_L = 5.00 mmol dm^{-3}, (*c*) c_{Cu} = 2.50, c_{Cd} = 10.00, c_L = 2.50 mmol dm^{-3}. 1, $[CuLH]^{2+}$; 2, $[CuL]^+$; 3, $[Cu_2L_2H_{-2}]$; 4, $[CuL_2H]^+$; 5, $[CuL_2]$; 6, $[CuCdLH_{-1}]^{2+}$; 7, $[CuCdLH_{-2}]^+$

data are consistent with the presence in solution of $[CuLH]^{2+}$, $[CuL]^+$, $[Cu_2L_2H_{-2}]$, $[CuL_2H]^+$, and $[CuL_2]$ complexes. If a monomeric species of the type $[CuLH_{-1}]$, previously proposed in the literature,[9] is introduced into the calculations, it is rejected.

The successive treatment of potentiometric data from titrations with $c_{Cu} > c_L$ (pH < 5.6—5.8) indicated that other species must be considered in addition to those previously listed: the numerical refinement has suggested the presence of homobinuclear species of the type $[Cu_2LH_{-1}]^{2+}$ (*e.g.*, when taking into account $c_{Cu} = 8 \times 10^{-3}$ and $c_L = 3 \times 10^{-3}$ mol dm^{-3}, the formation of $[Cu_2LH_{-1}]^{2+}$ with respect to the total ligand concentration is 28.7% at pH 5.7).

Cadmium–L-Histidylglycine System.—The study of the Cd^{II}–L system has confirmed the general behaviour of Cd^{II}–peptide complexes, which are formed without deprotonation of the peptide group. The numerical refinement is consistent with the presence in solution of the $[CdLH]^{2+}$, $[CdL]^+$, and $[CdL_2]$ species. Their stability constants are given in Table 3.

Copper–Cadmium–L-Histidylglycine System.—The experimental alkalimetric titration curves obtained in the presence of Cu^{II}, Cd^{II}, and the ligand significantly diverge, at pH > 5, from those calculated on the basis that only binary species are present. We have explained this discrepancy in terms of binuclear complex formation. The refinement with SUPERQUAD was performed both by considering as variable only the stability constants of the mixed-metal species and by allowing the variation of the stability constants of all Cu^{II} complexes (*i.e.*, binary and ternary), in order to avoid the small error in the values obtained for relevant binary complexes strongly affecting the values calculated successively for ternary species. The agreement we found between the values determined with the two different approaches is very satisfactory both for binary and ternary complexes. The heterobinuclear complexes present in

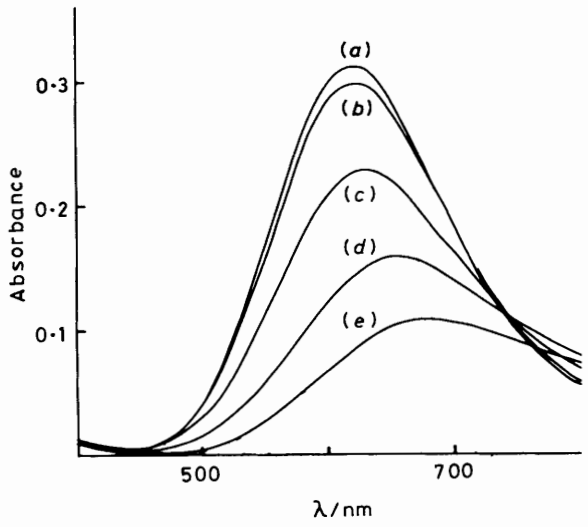


Figure 2. Visible spectra (c_{Cu} = 3.50, c_L = 3.60 mmol dm^{-3}) recorded at pH (*a*) 8.06, (*b*) 6.84, (*c*) 6.09, (*d*) 5.47, (*e*) 4.25; optical path length = 1 cm

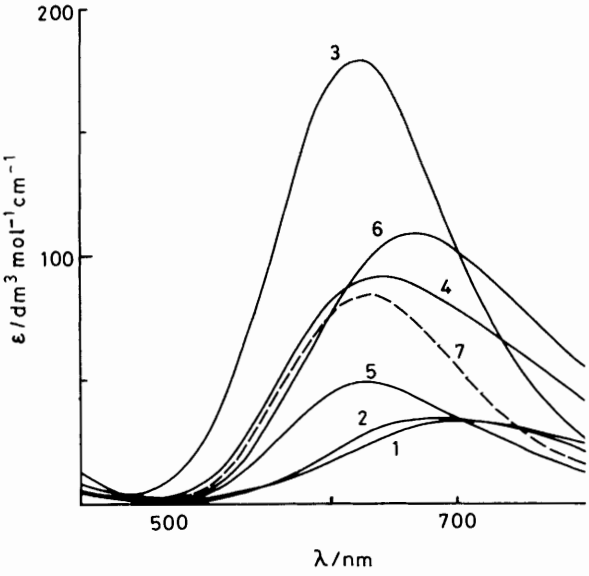


Figure 3. Visible spectra for the different species containing Cu^{II}: 1, $[CuLH]^{2+}$; 2, $[CuL]^{+}$; 3, $[Cu_2L_2H_{-2}]$; 4, $[CuL_2]$; 5, $[CuL_2H]^{+}$; 6, $[Cu_2LH_{-1}]^{2+}$; 7, $[CuCdLH_{-1}]^{2+}$

solution are of the type $[CuCdLH_{-1}]^{2+}$ and $[CuCdLH_{-2}]^{+}$. With suitable stoicheiometric reagent ratios (1:1:2) some evidence was obtained for a species $[CuCdL_2]^{2+}$ [log β_{1120} = 16.2(3)], whose relative formation did not exceed 5%. The values of the stability constants for the mixed-metal species are listed in Table 3.

Distribution diagrams, such as those given in Figure 1, allowed us to choose the experimental conditions (reagent concentration ratios and pH of solutions) in order to obtain high percentage formation of some selected species. So the spectra of the principal complexes present in solution were calculated by refining the visible spectrophotometric data from solutions in which the different species in turn were predominant. Examples of spectra which can be recorded on a solution containing Cu^{II} and hisgly with increasing pH are given in Figure 2: the shift of $\lambda_{max.}$ towards shorter wavelengths confirms the progressive increase of co-ordinated nitrogen-donor atoms per Cu^{II} ion. The spectra of the most relevant Cu^{II} complexes are given in Figure 3. The errors in the ε values (3σ) are ±3—4% for complexes such as $[CuL]^{+}$, $[CuL_2]$, and $[Cu_2L_2H_{-2}]$, while for the other species the uncertainties in ε can reach ±10%.

On the basis of distribution diagrams, the experimental conditions of the calorimetric measurements were chosen in order to obtain, for the desired species, the largest concentration difference in the solution before and after the titrant addition. The concentration of all the species were in turn predominant in the various measurements, so that more significant separations were obtained of the single contribution of the species to the total measured heat. In this way the enthalpies of complex formation using the computer programs were obtained with good accuracy. The values of the thermodynamic parameters are collected in Table 3 together with their probable errors (3σ).

Discussion

Potentiometric Data.—The complexation scheme we found for the Cu–hisgly system is in fairly good agreement with those already reported in the literature.[6,9,11] In contrast to other peptides such as glycyl-L-histidine or β-alanyl-L-histidine, in which the L-histidyl residue is not allowed to co-ordinate the Cu^{II} ion histamine-like, hisgly is able to form complexes through its free histamine residue. As regards deprotonated species there is disagreement in the literature about the existence of monomeric[9] (101 − 1) and dimeric[6,10,11] (202 − 2) complexes. According to Boggess and Martin[10] the dimerization constant is such that the dimer:monomer concentration ratio is 10 (at pH 7.9) at 10^{-4} mol dm^{-3} dimer and 100 at 0.01 mol dm^{-3} dimer. The refinement of our experimental data by SUPERQUAD indicates the formation of a relevant dimeric complex but it does not give a definite value for the monomer stability constant. Since our study was performed in the range 2.5—6 mmol dm^{-3} Cu^{II}, the formation of the 101 − 1 species should be almost negligible and this fact justifies the results obtained by the numerical refinement.

In the presence of an excess of Cu^{II} to ligand, two copper(II) ions per molecule can be bound. The stoicheiometry is the same as previously found with β-alanyl-L-histidine[18] and suggests that two separate co-ordinating centres may be effective in histidine-containing dipeptides. Binuclear complex formation also takes place with an excess of hetero ion (Cd^{II}), with the same metal ion to ligand stoicheiometric ratio.

Calorimetric and Spectrophotometric Data.—As regards the $[CuL]^{+}$ and $[CuL_2]$ species, the stepwise enthalpy values of Table 3 (−45.8 and −42 kJ mol^{-1}) are close to the values of −50.6 and 42.2 kJ mol^{-1}, previously found for the corresponding Cu–histamine complexes.[25] This is in agreement with the presence of copper–(amine) and –N(imidazole) bonds in the hisgly complexes.[8] It can also be observed, in support of this conclusion, that the calculation of the covalent and the electrostatic part of the enthalpy, following the treatment of Degischer and Nancollas,[26] leads to electrostatic contributions near zero (4 and 0 kJ mol^{-1}) compared with the covalent contribution of −50 and −42 kJ mol^{-1} for $[CuL]^{+}$ and $[CuL_2]$, respectively.

The present spectrophotometric data are in accordance with the above conclusions. Nevertheless it must be noted that, while very similar values have been obtained for hisgly and histamine in the 1:1 species ($\lambda_{max.}$ 677 and 681 nm; $\varepsilon_{max.}$ 35 and 35 dm^3 mol^{-1} cm^{-1}), different values of $\lambda_{max.}$ have been obtained for the 1:2 species: 640 and 598 nm for hisgly and histamine, respectively. Although the log (K_1/K_2) value appears too low in this case (it is even lower than that for copper–histamine), this

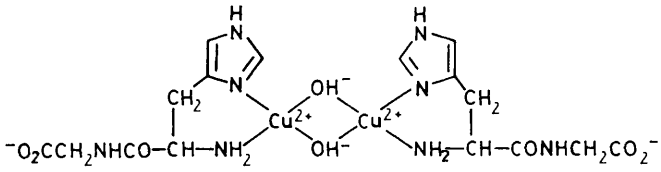
fact might be explained by assuming that one of the four nitrogen atoms co-ordinates the metal ion in an axial position in the hisgly complex.[27]

The presence of metal–carboxylate bonds in the $[CuL]^+$ complex species can be investigated considering the entropy values in the literature, and passing from the copper–acetate 1:1 complex (where complete neutralization of the electric charge of the carboxylate takes place) to the copper–histamine complex (in which copper–carboxylate interactions are absent). The following values are reported (J K^{-1} mol^{-1}): acetate, 42.7;[28] histidinate, 29.3;[29] hisgly, 15.5 (Table 3); histamine, −5.9.[29] These data appear to support a possible, weak metal–carboxylate interaction in the hisgly complex. It can also be noted that the entropy value of $[CuL_2]$ [reaction (5) of Table 3] is approximately equal and opposite in sign to that of $[CuL]^+$ (−17 *vs.* +15.5 J K^{-1} mol^{-1}), as though a solvent-structuring process takes place, when the second ligand enters the complex, about equal to the solvent-destructuring process for the first step. It is possible that the second ligand detaches from the metal, for steric reasons, the carboxylate group bound in the first step.

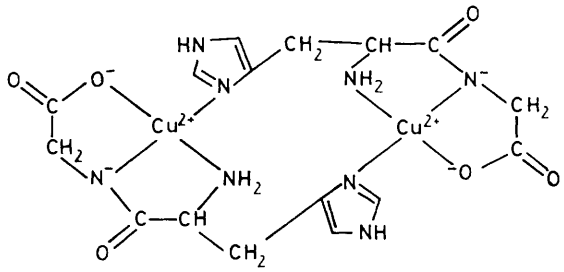
The visible spectrum of $[CuLH]^{2+}$ is quite similar to that of $[CuL]^+$ ($\lambda_{max.}$ 675 and 677 nm; $\varepsilon_{max.}$ 36 and 35 dm^3 mol^{-1} cm^{-1} respectively). These data are in agreement with a histamine-like co-ordination of copper in $[CuLH]^{2+}$, and with the protonation of the carboxylate group. The thermodynamic results also support this conclusion. An entropy value of 47 J K^{-1} mol^{-1} is given for reaction (6) in Table 3, *versus* 15.5 for $[CuL]^+$, although reaction (6), for stoicheiometric reasons, is entropically less favoured than the formation of $[CuL]^+$. A charge-neutralization process should then take place in the formation of $[CuLH]^{2+}$. On the other hand, the protonation of a nitrogen atom of histidylglycinate (ΔH° −41.4 or −31.8 kJ mol^{-1}, see Table 3) together with a copper–carboxylate bond (ΔH° = 5.2 kJ mol^{-1})[25] cannot explain the enthalpy value of −55 kJ mol^{-1} for reaction (6) in Table 3. The protonation of the carboxylate is then suggested by the experimental data for $[CuLH]^{2+}$. It must also be noted that a pK value of 3.26 results for the dissociation of –COOH in $[CuLH]^{2+}$, in comparison with 2.62 in the uncomplexed ligand. This fact can be justified by considering the presence of a $-NH_3^+$ group when the first proton is removed from the –COOH of the uncomplexed ligand, together with the remarkable inductive effect of $-NH_3^+$, even at a long distance. Such inductive effects are less important in the complexed ligand.

On the basis of spectrophotometric and thermodynamic data, it is not possible to arrive at definitive conclusions in the case of $[CuL_2H]^+$. The thermodynamic measurements give an entropy value near zero for the reaction: $Cu^{2+} + 2L^- \longrightarrow [CuL_2]$, *versus* 56 J K^{-1} mol^{-1} for the reaction: $Cu^{2+} + 2L^- + H^+ \longrightarrow [CuL_2H]^+$, although the latter is entropically less favoured than the former, for stoicheiometric reasons. Similar considerations to those above for $[CuLH]^{2+}$ could suggest a histamine-like interaction by both the hisgly groups with the metal ion; in this case, together with the protonation of the carboxylate. The spectrum of $[CuL_2H]^+$, on the other hand, is similar to that of $[Cu(hist)_2H]^{3+}$ (hist = histamine, $\lambda_{max.}$ 630 and 633 nm; $\varepsilon_{max.}$ 49 and 43 dm^3 mol^{-1} cm^{-1}) and is different from that of $[CuL_2]$ ($\lambda_{max.}$ = 598 nm, $\varepsilon_{max.}$ = 92 dm^3 mol^{-1} cm^{-1}).

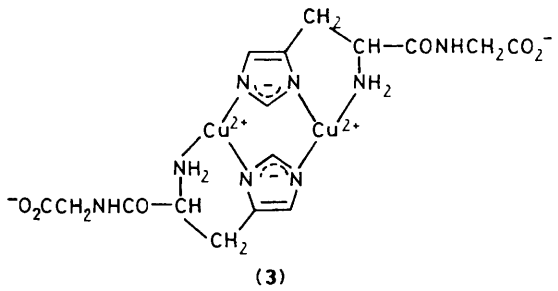
The protonation of an amine group in $[CuL_2H]^+$ together with an interaction of copper with the imidazole nitrogen and the carboxylate are shown to be possible by CPK models, although the formation of such a ring appears unlikely from a thermodynamic point of view. It must also be noted that this structure is in agreement with the pH range of formation of the species $[CuL_2H]^+$, which is higher than that of $[CuLH]^{2+}$ (see Figure 1).



(1)



(2)



(3)

Figure 4. Structures proposed for $[Cu_2L_2H_{-2}]$

A spectrum with $\lambda_{max.}$ = 669 nm and $\varepsilon_{max.}$ = 112 dm^3 mol^{-1} cm^{-1} has been obtained for the species $[Cu_2LH_{-1}]^{2+}$. It shows, compared with the spectrum of $[CuL'H_{-1}]$ (HL' = glycylglycine), higher values of $\lambda_{max.}$ and $\varepsilon_{max.}$. Both these effects are in accordance with the addition of a second copper(II) ion to an imidazole nitrogen, thus suggesting a copper–glycylglycine and a copper–imidazole nitrogen bond for the two metal ions in the species $[Cu_2LH_{-1}]^{2+}$. This conclusion is partially supported by the thermodynamic results. An enthalpy value of 2.8 kJ mol^{-1} was found for the formation of $[CuL'H_{-1}]$,[30] while a value of −29.0 kJ mol^{-1} resulted for the Cu^{II}–imidazole association.[31] The sum of these values is −26 kJ mol^{-1}, *versus* −19 kJ mol^{-1} for reaction (8) in Table 3.

Structures (**1**) and (**2**) of Figure 4 have been proposed previously for the species $[Cu_2L_2H_{-2}]$.[8,11] Structure (**3**) also appears possible, in accordance with the study of Mori *et al.*[32]

Considering reactions (4) and (9) of Table 3, together with structures (**1**) and (**2**) of Figure 4, it must be taken into account that Cu^{II}–carboxylate bond formation with the displacement of a water molecule from the cation has a negligible influence on enthalpy.[33] Reaction (9) in comparison with reaction (4) [see structures (**1**) and (**2**)] doubles the bonds of copper with the amine and the imidazole nitrogen. Moreover, two exchange reactions of the type $CONH + Cu^{2+} \longrightarrow CONCu^+ + H^+$ take place in the formation of structure (**2**), while an additional enthalpy variation for two ionizations of the type $H_2O \longrightarrow H^+ + OH^-$ is present for structure (**1**). As equal enthalpy values have been found for reactions (4) and (9) of Table 3 (−45.8 ± 0.6 and −46 ± 2 kJ mol^{-1} respectively), the conclusion should be drawn that the enthalpy of formation of two more bonds of copper with the amine and the imidazole nitrogen in reaction (9) is counterbalanced by the double

deprotonation reaction, implying that the enthalpy for the double deprotonation reaction is *ca.* 46 kJ mol^{-1}. The value of 23 kJ mol^{-1} so obtained for one deprotonation process in the formation of $[Cu_2L_2H_{-2}]$ is far from 57 kJ mol^{-1} for the water deprotonation [structure (**1**)], while it is closer to the value of 29 kJ mol^{-1} determined previously[34] for the exchange reaction: $CONH + Cu^{2+} \longrightarrow CONCu^{+} + H^{+}$. Structure (**2**) rather than structure (**1**) is thus indicated as more probable by the thermodynamic data.

The deprotonation of the pyrrole nitrogen is considered in structure (**3**) rather than of the peptide nitrogen, as in structure (**2**). As the enthalpy values for these deprotonation processes are probably similar,[35] it not possible to distinguish between structures (**2**) and (**3**) on the basis of the enthalpy data. Nevertheless, it can be observed that a double copper–carboxylate charge neutralization takes place in structure (**2**), while this interaction seems very weak in structure (**3**) (see the discussion of the species $[CuL]^{+}$). For this reason structure (**2**) should be entropically more favoured than structure (**3**). Moreover, two six-membered rings are present in (**3**), compared with four five-membered rings in (**2**), so that the latter should be more enthalpy-favoured than the former.

Close similarity observed between the visible spectra of a solution of Cu^{II} with hisgly and a solution of Cu^{II} with imidazole and glycylglycine, both at the same degree of neutralization, led Aiba *et al.*[8] to indicate structure (**2**) as the most probable. The spectrum calculated in the present study for the species $[Cu_2L_2H_{-2}]$ ($\lambda_{max.}$ = 619 nm; $\varepsilon_{max.}$ = 179 dm^3 mol^{-1} cm^{-1}) is in good accordance with that reported in the cited paper.[8]

The enthalpy value of -22.2 kJ mol^{-1} in Table 3 for the formation of $[CdL]^{+}$ is close to that (-22.6 kJ mol^{-1}) found for Cd with histamine,[36] in accordance with a histamine-like interaction for this metal also. An analogous structure is also suggested by the experimental data for $[CdL_2]$.

For the species $[CdLH]^{2+}$ [reaction (12) of Table 3] an entropy value was found close to that of $[CdL]^{+}$ (9 and 12 J K^{-1} mol^{-1}, respectively). Quite different values, on the other hand, were found in the case of copper (47 J K^{-1} mol^{-1} for $[CuLH]^{2+}$ and 15.5 J K^{-1} mol^{-1} for $[CuL]^{+}$), which indicate a possible proton-carboxylate neutralization in the species $[CuLH]^{2+}$. Considering also the pH at which $[CdLH]^{2+}$ is formed (*ca.* 5—6), the protonation of the amine nitrogen, with no participation of the carboxylate, seems probable in this species.

For the complex species $[CuCdLH_{-1}]^{2+}$, a spectrum similar to that of $[CuL'H_{-1}]$ has been calculated ($\lambda_{max.}$ 630 and 639 nm; $\varepsilon_{max.}$ 82 and 83 dm^3 mol^{-1} cm^{-1}). A glycylglycine-like interaction for copper together with a cadmium–imidazole nitrogen bond may then be inferred from this comparison. The enthalpy data are in accordance with this conclusion. In fact the value calorimetrically determined for the formation of $[CuL'H_{-1}]$ (+2.8 kJ mol^{-1}),[30] if added to the value found for the cadmium–imidazole association (-20.4 kJ mol^{-1}),[37] gives -17.6 kJ mol^{-1}, which is fairly close to the value of -15 ± 2 kJ mol^{-1} of reaction (13).

The pK^{H} value (7.95) for reaction $[CuCdLH_{-1}]^{2+} \longrightarrow [CuCdLH_{-2}]^{+} + H^{+}$ is more than one order of magnitude smaller than pK^{H} for $[CuL'H_{-1}]$[38] and suggests a greater tendency to hydrolysis for $[CuCdLH_{-1}]^{2+}$.

References

1 A. P. Brunetti, M. C. Lim, and G. H. Nancollas, *J. Am. Chem. Soc.*, 1968, **90**, 5120.
2 R. J. Sundberg and R. B. Martin, *Chem. Rev.*, 1974, **74**, 471.
3 P. Z. Neumann and A. Sass-Kortsak, *J. Clin. Invest.*, 1967, **46**, 646.
4 B. Sarkar and Y. Wigfield, *Can. J. Biochem.*, 1968, **46**, 601.
5 G. Arena, E. Rizzarelli, and B. Sarkar, *Inorg. Chim. Acta*, 1979, **37**, L555.
6 I. Sóvágó, E. Farkas, and A. Gergely, *J. Chem. Soc., Dalton Trans.*, 1982, 2159.
7 E. Farkas, I. Sóvágó, T. Kiss, and A. Gergely, *J. Chem. Soc., Dalton Trans.*, 1984, 611.
8 H. Aiba, A. Yokoyama, and H. Tanaka, *Bull. Chem. Soc. Jpn.*, 1974, **47**, 136.
9 G. Brookes and L. D. Pettit, *J. Chem. Soc., Dalton Trans.*, 1975, 2112.
10 R. K. Boggess and R. B. Martin, *J. Inorg. Nucl. Chem.*, 1975, **37**, 1097.
11 R. P. Agarwal and D. D. Perrin, *J. Chem. Soc., Dalton Trans.*, 1975, 268.
12 J. S. Richardson, K. A. Thomas, B. H. Rubin, and D. C. Richardson, *Proc. Natl. Acad. Sci. USA*, 1975, **72**, 1349.
13 J. Heut and E. Vilkas, *Inorg. Chim. Acta*, 1984, **91**, 43.
14 P. Amico, G. Arena, P. G. Daniele, G. Ostacoli, E. Rizzarelli, and S. Sammartano, *Environ. Inorg. Chem.*, 1985, 285.
15 D. R. Williams (ed.), 'An Introduction to Bio-Inorganic Chemistry,' C. C. Thomas, Illinois, 1976.
16 M. L. D. Touche and D. R. Williams, *J. Chem. Soc., Dalton Trans.*, 1976, 1355.
17 P. Amico, P. G. Daniele, G. Arena, G. Ostacoli, E. Rizzarelli, and S. Sammartano, *Inorg. Chim. Acta*, 1979, **35**, L383.
18 P. G. Daniele, P. Amico, and G. Ostacoli, *Inorg. Chim. Acta*, 1982, **66**, 65.
19 P. Gans, A. Sabatini, and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1985, 1195.
20 A. Sabatini and A. Vacca, personal communication.
21 S. Sammartano, personal communication.
22 A. Vacca, personal communication.
23 G. Arena, R. Calì, E. Rizzarelli, and S. Sammartano, *Thermochim. Acta*, 1976, **16**, 315.
24 C. F. Baes, jun., and R. E. Mesmer, 'The Hydrolysis of Cations,' Wiley, New York, 1976.
25 G. Arena, R. Cali, V. Cucinotta, S. Musumeci, E. Rizzarelli, and S. Sammartano, *J. Chem. Soc., Dalton Trans.*, 1984, 1651.
26 G. Degischer and G. H. Nancollas, *J. Chem. Soc. A*, 1970, 1125.
27 E. J. Billo, *Inorg. Nucl. Chem. Lett.*, 1974, **10**, 613.
28 R. Aruga, *Ann. Chim. Rome*, 1974, **64**, 659.
29 J. L. Meyer and J. E. Bauman, jun., *J. Am. Chem. Soc.*, 1970, **92**, 4210.
30 A. Gergely and I. Nagypal, *Mag. Kem. Foly.*, 1976, **82**, 442.
31 G. Arena, R. P. Bonomo, G. Impellizzeri, R. M. Izatt, J. D. Lamb, and E. Rizzarelli, *Inorg. Chem.*, 1987, **26**, 795.
32 W. Mori, A. Nakahara, and Y. Nakao, *Inorg. Chim. Acta*, 1979, **37**, L507.
33 G. H. Nancollas, 'Interactions in Electrolyte Solutions,' Elsevier, Amsterdam, 1966.
34 E. W. Tipping and H. A. Skinner, *J. Chem. Soc., Faraday Trans. 1*, 1972, 1764.
35 H. Sigel and R. B. Martin, *Chem. Rev.*, 1982, **82**, 385.
36 S. N. R. V. Schalien, *Suom. Kemistil. B*, 1959, **32**, 148.
37 F. Marsicano and R. D. Hancock, National Institute for Metallurgy, Johannesburg, South Africa, Report no. 1764, 1975.
38 P. G. Daniele, O. Zerbinati, R. Aruga, and Giorgio Ostacoli, unpublished work.

Received 9*th April* 1987; *Paper* 7/607