Thermodynamic and Spectroscopic Study of Copper(II)–Glycyl-L-histidylglycine Complexes in Aqueous Solution

Pier G. Daniele,* Orfeo Zerbinati, Vincenzo Zelano and Giorgio Ostacoli
Universita di Torino, Dipartimento di Chimica Analitica, Via Pietro Giuria 5, 10125 Torino, Italy

The complexes formed in aqueous solution by copper(II) with glycyl-L-histidylglycine (L^-) have been extensively studied, by using potentiometry, calorimetry, visible spectrophotometry and circular dichroism techniques, with the aim of elucidating their type and probable structure. Only monomeric species are formed in the acidic range of pH ($[CuL]^+$, $[CuLH_{-1}]$, $[CuL_2]$ and $[CuL_2H_{-1}]^-$), while at higher pH (>8) polynuclear $[Cu_4L_4H_{-8}]^{4-}$ is formed in addition to $[CuLH_{-2}]^-$. The interpretation of all the experimental data is strongly consistent with a structure involving three nitrogen atoms co-ordinated to copper(II) for $[CuLH_{-1}]$ and $[CuL]^+$ and with the participation of a fourth nitrogen atom in $[CuLH_{-2}]^-$ and $[Cu_4L_4H_{-8}]^{4-}$. In the tetrameric species very probably there is a dissociation of the N(1)-pyrrole hydrogen ion, with consequent formation of imidazolate bridges.

It is well known that a histidyl residue in a peptide molecule strongly affects the type of copper(II) complexes formed in solution and their stability. The co-ordinating ability of imidazole towards copper(II) in addition to that of groups normally present in a dipeptide (amino, peptide and carboxylate groups) in some cases allows the formation of binuclear species (homo- and hetero-binuclear).[1,2] Copper(II) dimeric complexes, not formed by alkyl-substituted glycylglycines in the acidic to neutral region of pH, are very relevant species for dipeptides such as β-alanyl-L-histidine (L-carnosine)[1] or L-histidylglycine.[2] The positon of the histidyl residue in the peptide chain is critical in determining the type of complex(es) formed and its(their) stability in solution. Unlike these two peptides, glycyl-L-histidine does not form dimeric complexes with copper(II) in aqueous solution.[3–5]

As regards L-histidine-containing tripeptides, the most relevant copper(II) species in solution formed by glycylglycyl-L-histidine (gly-gly-his) is $[Cu(gly\text{-}gly\text{-}his)H_{-2}]^-$ with copper(II) co-ordinated by four nitrogen atoms [one amino, one N(3)-imidazole and two deprotonated peptide nitrogens,[6–8] while the structure of glycyl-L-histidylglycine (gly-his-gly) does not seem to favour the dissociation of both peptide groups, when complexing copper(II),[8] and the resulting predominant species is $[Cu(gly\text{-}his\text{-}gly)H_{-1}]$, having a copper(II) ion co-ordinated by three nitrogen atoms.[6,8] On the basis of a pH-static technique, Österberg and Sjöberg,[9] in addition to the above relevant species, suggested the presence in solution of polymeric species involving carboxylate bridges.

Since there are no literature values for thermodynamic parameters (ΔG°, ΔH° and ΔS°) of copper(II) complexes with glycyl-L-histidylglycine and little spectroscopic information is available, we have investigated the binary copper(II)–gly-his-gly system by potentiometry, calorimetry, visible spectrophotometry and visible circular dichroism (CD) techniques.

Experimental

Chemicals.—The purity of glycyl-L-histidylglycine $[H_3L]^{2+}$, (Sigma product) was checked by ion chromatography and by alkalimetric titrations, after drying in a vacuum. Copper(II) nitrate stock solutions were prepared and standardized as previously reported.[1] All the solutions were prepared by using twice-distilled water. The ionic strength was adjusted to 0.1 mol dm^{-3} by addition of KNO_3.

Electromotive Force Measurements.—Potentiometric measurements were performed at 25 °C and ionic strength $I = 0.1$ mol dm^{-3} (KNO_3) with a Metrohm 605 potentiometer equipped with glass and calomel electrodes. The electrode couple was calibrated in $-\log[H^+]$ units (pH) by titrating nitric acid (4–10 mmol dm^{-3}) with standard, carbonate-free, potassium hydroxide. The ionic composition and the ionic strength of the calibrating solution were the same as those of the solution being examined. The concentration of copper(II) ranged from 1 to 10 mmol dm^{-3} with metal to ligand ratios from 3:1 to 1:3.

Visible Spectrophotometric and Circular Dichroism Measurements.—The visible spectrophotometric determinations were carried out with a Spectracomp 601 Carlo Erba spectrophotometer and visible circular dichroism was recorded by a J-600 JASCO spectropolarimeter, from 400 to 800 nm, under the same experimental conditions as for potentiometric measurements. The solutions being examined were transferred from the potentiometric to an optical cell using a peristaltic pump. The metal concentrations and metal to ligand ratios were the same as for the potentiometric determinations (only 1:1 $c_{Cu}:c_L$ ratios were studied by circular dichroism, the c values being analytical concentrations).

Calorimetric Measurements.—Calorimetric experiments were performed at 25.00 ± 0.02 °C ($I = 0.1$ mol dm^{-3}) using a LKB 2107 microcalorimeter (batch calorimeter with heat-flux measuring system). The accuracy and reproducibility of the system and the experimental procedure are the same as previously reported.[2]

Data Analysis and Calculations.—The values of the protonation constants (pK^{H_i}) reported in this paper can be assigned to the carboxylate group, N(3) of imidazole and amino nitrogen. Up to pH 10 the dissociation of peptide and N(1)-pyrrole hydrogens are negligible in the absence of metal ions. The stability constants are expressed by the general equation $\beta_{pqr} = [Cu_pL_qH_r]/[Cu]^p[L]^q[H]^r$. The values of $\log \beta_{pqr}$ were refined by means of the NSTACO[10] program which minimizes the error squares sum on electromotive force values and takes into account eventual variations of ionic strength among and/or during titrations.[11] Spectrophotometric and circular dichroism data were analysed by the MOLEX[12] program which calculates the values of the molar absorption coefficients (ε_λ) or of

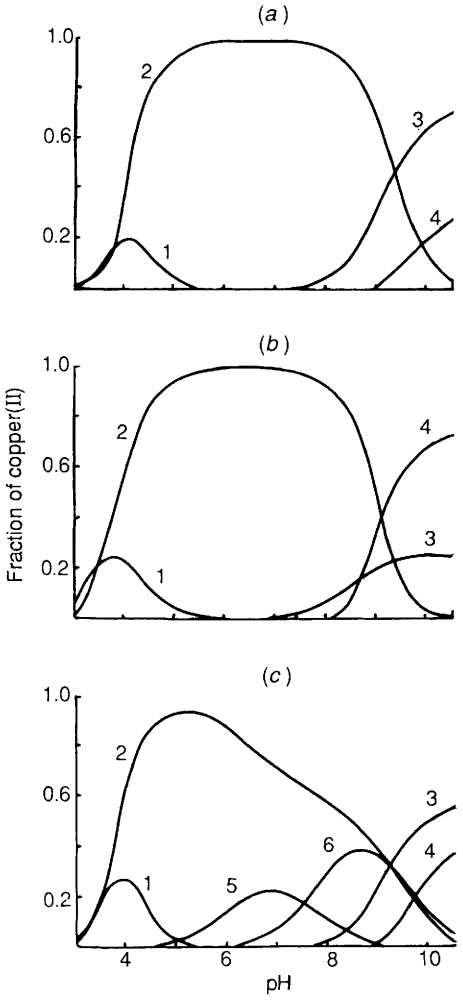

Fig. 1 Species distribution *vs.* pH referred to total copper(II), in the system copper(II)–glycyl-L-histidylglycine: (*a*) $c_{Cu} = c_L = 2.0$, (*b*) $c_{Cu} = c_L = 10.0$ and (*c*) $c_{Cu} = 3.0$, $c_L = 6.0$ mmol dm^{-3}. Species: 1, $[CuL]^+$; 2, $[CuLH_{-1}]$; 3, $[CuLH_{-2}]^-$; 4, $[Cu_4L_4H_{-8}]^{4-}$; 5, $[CuL_2]$; 6, $[CuL_2H_{-1}]^-$

molecular ellipticities ($[\theta]_\lambda = \psi_\lambda/cl \times 100$, where ψ_λ is ellipticity in °, c concentration in mol dm^{-3} and l is the pathlength in cm.) and therefore of $\Delta\varepsilon$ of the complex species, using experimental data on absorbance or ellipticity, respectively, and known values of stability constants. The calorimetric data were refined by the ES5CM [13] program. In all calculations the hydrolysis of copper(II) ion was taken into account.[14]

Results and Discussion

The alkalimetric titration of an equimolar mixture of copper(II) and glycyl-L-histidylglycine gives a curve with a sharp break-point and two well defined buffer regions. The first buffer zone corresponds to deprotonation of the N(3)-imidazole, amino and one peptide (N-terminal) groups. The refinement of potentiometric data up to pH 7 indicates that only mononuclear species are present in solution [even in the presence of excess of copper(II)] of the type $[CuL]^+$ and $[CuLH_{-1}]$. If base addition is continued, further dissociation of hydrogen ions occurs, probably from the N(1)-pyrrole hydrogen of the imidazole ring or from the second peptide group. It is more difficult to interpret the potentiometric readings obtained in the second buffer region. The presence of polynuclear species has been suggested by several authors,[3,6,8,9] therefore we investigated copper(II) concentrations ranging from 1 to 10 mmol dm^{-3}. An examination of visible spectrophotometric data recorded on solutions containing equimolar mixtures of copper(II) and tripeptide ligand, at a pH value of about 10.5 (at this pH the complex formation in the latter buffer region can be considered complete), shows that the value of λ_{max} is slightly (but significantly) shifted towards shorter wavelengths with increasing copper(II) concentration: $\lambda_{max} = 568$ nm for $c_{Cu} = 1$ mmol dm^{-3}, 554 nm for $c_{Cu} = 10$ mmol dm^{-3}, with intermediate values for copper(II) concentrations within this range. This experimental evidence indicates that with respect to $[CuLH_{-1}]$ another nitrogen donor atom takes part in co-ordination, but also that more than one species is formed, with slightly different co-ordination modes. The refinement of potentiometric data by the NSTACO program indicated that the minimum error is reached under the assumption that monomeric $[CuLH_{-2}]^-$ and tetrameric $[Cu_4L_4H_{-8}]^{4-}$ species are present in solution at the same time. The distribution diagrams *vs.* pH clearly point out the relevance of the complex $[CuLH_{-1}]$ and the relative concentrations of $[CuLH_{-2}]^-$ and $[Cu_4L_4H_{-8}]^{4-}$ as a function of total copper(II) concentration [Fig. 1(*a*) and (*b*)].

If solutions containing an excess of metal ion (up to $c_{Cu}:c_L = 3:1$) are titrated to precipitation (pH 5.6–5.8) no binuclear species were found, indicating the high stability of $[CuLH_{-1}]$ and the lack of co-ordination of the second peptide group, in the acidic to neutral range of pH. The addition of a ligand excess to the solution, on the other hand, gives rise to the formation of $[CuL_2]$ and $[CuL_2H_{-1}]^-$ species, even in the presence of $[CuLH_{-1}]$, $[CuLH_{-2}]^-$ and $[Cu_4L_4H_{-8}]^{4-}$ [see Fig. 1(*c*)].

The values of all the stability constants for protonation and complex formation are collected in Table 1. There is fairly good agreement between the formation constants of Gergely and co-workers[8] and ours; however, the stoichiometry of the species formed from $[CuLH_{-1}]$, upon further deprotonation, was not defined earlier.[8]

The calorimetric data were analysed by considering all the complexes of Table 1. The values determined for $\Delta G°$, $\Delta H°$ and $\Delta S°$ are listed in the same table.

Visible spectra obtained for this system at different pH values are reported in Fig. 2(*a*). Before the inflection point, λ_{max} undergoes a blue shift, with increase in pH, up to 606 nm. In the second buffer region there is a further blue shift of up to 568–554 nm, which is dependent on the total copper(II) concentration. This significant shift, accompanied by dissociation of one H^+ per Cu^{2+}, suggests the involvement of another nitrogen atom in co-ordination, in accordance with the reports of Aiba *et al.*,[6] Gergely and co-workers[8] and Morris and Martin (for glycyl-L-histidine[3]). The same blue shift with increasing pH is observed in CD measurements [Fig. 3(*a*)].

Both visible spectrophotometric and CD measurements, recorded at different values of total reagent concentrations and pH, were analysed by means of the MOLEX program [12] (taking into account known values of stability constants, Table 1), in order to obtain for each complex the values of the molar absorption coefficients ε_λ and molecular ellipticities $[\theta]_\lambda$ (and therefore of $\Delta\varepsilon$). The results of these calculations are shown in Figs 2(*b*) and 3(*b*), respectively.

The most important species in solution, $[CuLH_{-1}]$, is formed by deprotonation of one peptide group. If the reactions $Cu^{2+} + \text{gly-DL-alaO}^- \rightleftharpoons [Cu(\text{gly-DL-alaO})H_{-1}] + H^+$ (log $\beta = 1.55$, $\Delta H° = -3$ kJ mol^{-1})[15] and $Cu^{2+} + \text{Him} \rightleftharpoons [Cu(\text{Him})]^{2+}$ (log $\beta = 4.07$, $\Delta H° = -29$ kJ mol^{-1})[16] are taken into account (alaO = alaninate, Him = imidazole), both the value of log β_{11-1} and that of $\Delta H°$ for the same complex are consistent with the presence of a copper(II) ion co-ordinated by amino, dissociated peptide and N(3)-imidazole nitrogens.

The values of $\lambda_{max} = 606$ nm and $\varepsilon_{max} = 55$ dm^3 mol^{-1} cm^{-1} for $[CuLH_{-1}]$ [Fig. 2(*b*)] are in very good agreement with data of Gergely and co-workers and even with those of Morris and Martin[3] referred to copper(II) complexes of glycyl-L-histidine, while the values of $\lambda_{max} = 605$, $\Delta\varepsilon = 0.35$ and $\lambda_{max} = 494$ nm, $\Delta\varepsilon = -0.05$ dm^3 mol^{-1} cm^{-1} [Fig. 3(*b*)], obtained for CD spectrum agree well with those of Ueda *et al.*[17] The value of λ_{max} calculated for the visible spectrophotometric band, by the equation proposed by Billo,[18] if the co-ordinating groups are

Table 1 Values of the ligand protonation (as pK^{H_i}) and complex formation (as log β_{pqr}) constants in the system copper(II)–glycyl-L-histidylglycine, at 25 °C and I = 0.1 mol dm^{-3}, and of the thermodynamic parameters[a]

Reaction	pK^{H_i}	$-\Delta G^\circ$	$-\Delta H^\circ$	ΔS°
$H^+ + L^- \rightleftharpoons HL$	7.995(8)[b]	45.61(5)	40.5(4)	17(1)
$H^+ + HL \rightleftharpoons H_2L^+$	6.50(2)	37.1(2)	32.0(6)	17(2)
$H^+ + H_2L^+ \rightleftharpoons H_3L^{2+}$	3.08(3)	17.5(2)	0(2)	59(7)
	log β_{pqr}	$-\Delta G^\circ$	$-\Delta H^\circ$	ΔS°
$Cu^{2+} + L^- \rightleftharpoons [CuL]^+$	9.35(3)	53.3(2)	38(1)	51(4)
$Cu^{2+} + L^- \rightleftharpoons [CuLH_{-1}] + H^+$	5.66(1)	32.3(1)	31.5(6)	3(2)
$Cu^{2+} + L^- \rightleftharpoons [CuLH_{-2}]^- + 2H^+$	−3.70(4)	−21.0(3)	8(1)	−98(5)
$4Cu^{2+} + 4L^- \rightleftharpoons [Cu_4L_4H_{-8}]^{4-} + 8H^+$	−7.20(8)	−41.1(6)	14(2)	−185(7)
$Cu^{2+} + 2L^- \rightleftharpoons [CuL_2]$	15.96(7)	91.0(4)	104(2)	−42(8)
$Cu^{2+} + 2L^- \rightleftharpoons [CuL_2H_{-1}]^- + H^+$	8.32(5)	47.5(3)	61(1)	−45(5)

[a] ΔG° and ΔH° in kJ mol^{-1}, ΔS° in J K^{-1} mol^{-1}; 25 °C. [b] The errors in parentheses are $\pm 3\sigma$ in the last significant figures.

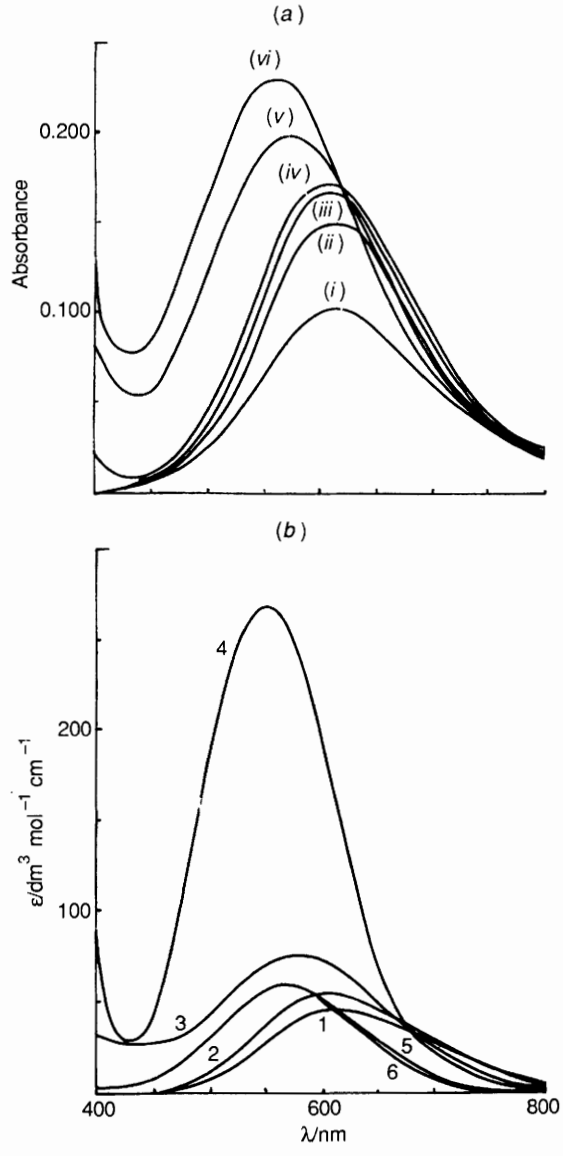

Fig. 2 Visible spectra (*a*) recorded for a solution containing $c_{Cu} = c_L$ = 3.0 mmol dm^{-3}, at pH 3.94 (*i*), 4.50 (*ii*), 6.18 (*iii*), 8.52 (*iv*), 9.60 (*v*) and 10.75 (*vi*); (*b*) calculated for different species (see Fig. 1) containing copper(II)

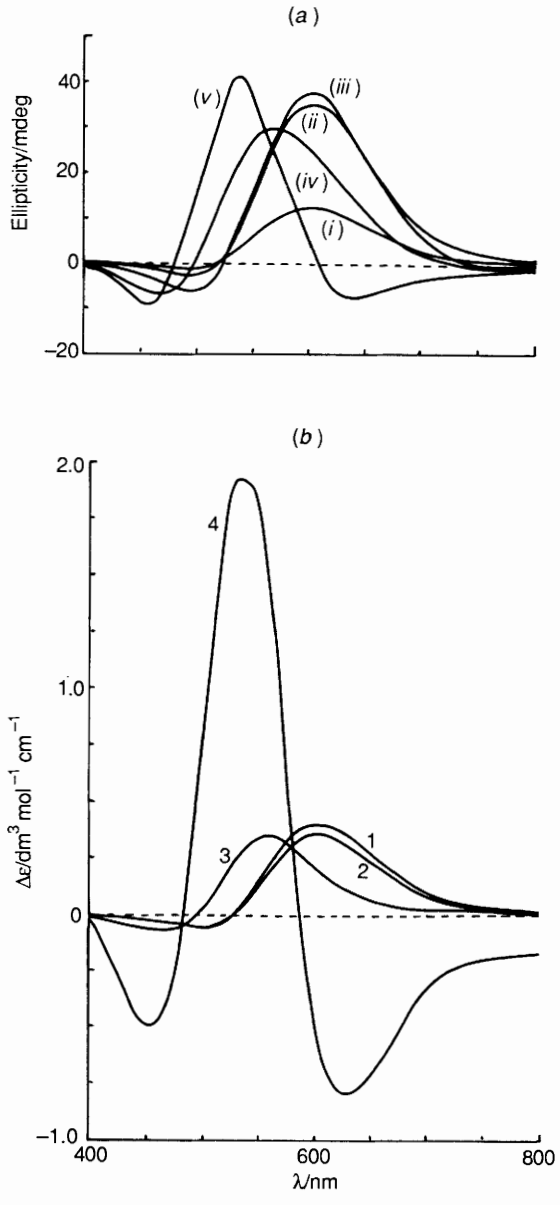

Fig. 3 Visible circular dichroism spectra (*a*) recorded on a solution containing $c_{Cu} = c_L$ = 4.0 mmol dm^{-3}, at pH 3.66 (*i*), 4.34 (*ii*), 7.50 (*iii*), 9.27 (*iv*) and 11.05 (*v*); (*b*) calculated for species containing 1:1 copper(II): ligand ratios: 1, $[CuL]^+$; 2, $[CuLH_{-1}]$; 3, $[CuLH_{-2}]^-$; 4, $[Cu_4L_4H_{-8}]^{4-}$. mdeg = millidegree

amino, dissociated peptide and N(3)-imidazole nitrogens in addition to a water molecule, is 599 nm, in good agreement with the experimental one.

A monodentate character, with co-ordination by only the amino group, in $[CuL]^+$ is scarcely consistent with the high value of the stability constant. The values of thermodynamic

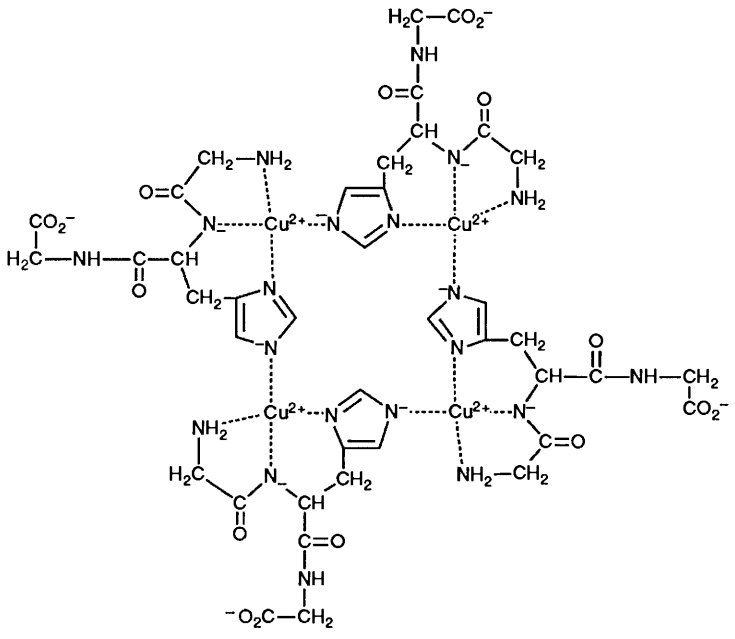

Fig. 4 Structure proposed for the tetrameric species $[Cu_4L_4H_{-8}]^{4-}$

parameters (Table 1) cannot be justified either by assuming a co-ordination only by the amino group or by only the N(3)-imidazole nitrogen. The fairly close values of ΔH° estimated for the $[CuL]^+$ and $[CuLH_{-1}]$ complexes, together with the significantly higher value of ΔS° evaluated for $[CuL]^+$ (with respect to $[CuLH_{-1}]$) may suggest that the two species have the same structure, involving protonation of the C-terminal carboxylate group in $[CuL]^+$. This hypothesis is well confirmed by the visible and CD spectra: in fact both these spectroscopic techniques give very similar spectra for $[CuL]^+$ and $[CuLH_{-1}]$ [Figs. 2(*b*) and 3(*b*)].

It seems likely that also the species having two ligand molecules are formed starting from the same structure as that of $[CuLH_{-1}]$. The more convincing hypothesis takes into account the reaction $[CuLH_{-1}] + HL \rightleftharpoons [CuL_2]$, with the second ligand (having the amino group protonated) co-ordinated through its N(3)-imidazole nitrogen. The total contribution to ΔH° can be estimated from the sum of ΔH° for the formation of $[CuLH_{-1}]$ (-31.5 kJ mol^{-1}, Table 1), $[Cu(Him)]^{2+}$ (-29 kJ mol^{-1})[16] and protonation of the amino group (-40.5 kJ mol^{-1}, Table 1). The resulting value of -101 kJ mol^{-1} may be considered in good agreement with that experimentally determined (-104 kJ mol^{-1}, Table 1). On this hypothesis, the value of λ_{max} calculated for this complex by using the equation of Billo[18] is 556 nm, which is in very good agreement with the experimental value of 559 nm. A similar structure may be proposed for $[CuL_2H_{-1}]^-$, with dissociation of the protonated amino group, in the ligand molecule bonded to copper(II) through its N^3-imidazole nitrogen. The visible spectra of these two complexes are very similar ($\lambda_{max} = 559$, $\varepsilon_{max} = 58$ and $\lambda_{max} = 556$ nm, $\varepsilon_{max} = 57$ dm^3 mol^{-1} cm^{-1} for $[CuL_2]$ and $[CuL_2H_{-1}]^-$, respectively) and the difference between ΔH° values calculated for these two species (-43 kJ mol^{-1}, Table 1) is close to the value obtained for protonation of the amino group.

As regards the complexes formed in the second buffer region, the probable participation of another nitrogen atom in co-ordination was previously suggested by several authors,[3,6,8] on the basis of spectroscopic evidence. Also the values of ΔH° estimated for the species $[CuLH_{-2}]^-$ and $[Cu_4L_4H_{-8}]^{4-}$, if compared with that calculated for $[CuLH_{-1}]$, are strongly consistent with a reaction involving the substitution of a hydrogen attached to a nitrogen donor by a copper(II) ion. The presence of only one species in the latter buffer region is not sufficient to explain both the trend in alkalimetric titrations and the shift of λ_{max} with varying copper(II) total concentration. The analysis of all experimental data is consistent with the presence of two (one monomeric and the other tetrameric) complexes. A similar tetrameric species for nickel(II) and glycyl-L-histidine has been proposed by Morris and Martin.[3]

The only way for one molecule of glycyl-L-histidylglycine to co-ordinate copper(II) with four nitrogen atoms implies the participation of amino, two deprotonated peptide and N(3)-imidazole nitrogens. Owing to the structure of the peptide the co-ordination of these four nitrogen atoms around copper(II) in the plane is not likely. The value of λ_{max} obtained for $[CuLH_{-2}]^-$ [577 nm, Fig. 2(*b*)] is much higher than that estimated by using the equation of Billo,[18] with the hypothesis that all nitrogens co-ordinate in the plane (540 nm). This difference can be explained by assuming that one nitrogen [probably N(3)-imidazole nitrogen] co-ordinates in an axial position. On the other hand, the value of $\lambda_{max} = 552$ nm [Fig. 2(*b*)] calculated for the tetrameric $[Cu_4L_4H_{-8}]^{4-}$ species suggests a configuration close to a planar co-ordination of all nitrogen atoms around each copper(II) ion, probably through the participation of amino, N(3)-imidazole, one deprotonated peptide and N(1)-deprotonated pyrrole nitrogens: in fact, if we assume that the contribution in Billo's equation due to the deprotonated N(1)-pyrrole nitrogen is the same as that of the deprotonated peptide nitrogen, the value estimated for λ_{max} is still 540 nm, which is close to that calculated for this polynuclear complex (552 nm). The structure proposed for the tetrameric complex is shown in Fig. 4, where the N_4 co-ordination of the four copper(II) ions is possible through the formation of imidazolate bridges.

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