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Microspeciation in the Copper(II)–L-Histidylglycine System. An ESR Study by the Two-Dimensional Computer Simulation Method

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Twelve ESR-active (and one inactive) copper(II) complexes of L-histidylglycine (HL) were characterized via their formation (micro)constants and ESR parameters obtained by two-dimensional ESR spectroscopic evaluation in aqueous solution. In strongly acidic media, the ligand is coordinated through its N-terminal donor groups: the complex $[CuLH_2]^{3+}$ involves monodentate imidazole binding, whereas $[CuLH]^{2+}$ involves bidentate ligation through the amino and imidazole N atoms. This histamine-like bonding mode also predominates in the isomers of $[CuL_2]$, formed at ligand excess near pH 7: in the major 4N isomer, both ligands occupy two equatorial sites, while in the 3N isomer, the second dipeptide is coordinated equatorially by the amino and axially by the imidazole groups. At above pH 3–4, deprotonation of the peptide group also starts: in ≈60% of the molecules of $[CuL]^+$, the peptide group is deprotonated, while in the minor isomer histamine-like coordination occurs. At higher pH, the active dimer $[Cu_2L_2H_{-2}]$, the mixed hydroxo complexes (the inactive $[Cu_2L_2H_{-3}]^-$ and the active $[CuLH_{-2}]^-$), and the bis complexes $[CuL_2H]^+$ and $[CuL_2H_{-1}]^-$ all involve tridentate equatorial ligation of the backbone by the amino and deprotonated peptide N and the carboxylate O atoms. In the active dimer, the neutral imidazole groups form bridges between $CuLH_{-1}$ units. In $[CuL_2H]^+$, the second ligand is bound equatorially via its imidazole group; in $[CuL_2H_{-1}]^-$, the L ligand occupies the fourth equatorial site and an axial site through its amino and imidazole N atoms, respectively.

Introduction

We recently developed a new electron spin resonance (ESR) simulation method for the study of multicomponent equilibrium systems of paramagnetic complexes.[1] Our two-dimensional approach considers the ESR intensity as a function of the magnetic field and the concentration data (the metal and ligand concentrations and the pH) varied in the course of ESR recordings, and it furnishes the pertinent formation constants in the mass-balance equations and the individual ESR parameters for all species simultaneously. The different paramagnetic species are most likely to have distinguishable spectra, as the ESR parameters are highly sensitive to alterations in the coordination. Consequently, our method can provide information on the speciation and coordination modes even in cases when traditional methods such as pH potentiometry or spectrophotometry fail to detect or distinguish certain species.

By pH potentiometry, for example, it is difficult to show complexes formed without proton uptake or loss. Moreover, in contrast with our method, which yields the parameters and formation microconstants of isomers of even nonpredominant species, pH potentiometry alone or in combination with spectrophotometry cannot distinguish between coexisting modes of different coordination of a given complex. This may be a particularly difficult problem when alternative structures of comparable stability are competing, as in the complexation of L-histidylglycine to copper(II). In this case, the N-terminal position of the imidazole group makes histamine-like binding a strong rival of the usual coordination modes by the backbone donor groups. Additionally, copper(II) may induce deprotonation of the imidazole group in alkaline solution.

The possible complexity of the competing processes and the limited selectivity of the techniques applied might explain why general agreement has not yet been achieved as regards

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the speciation and molecular structures in the copper(II)−L-histidylglycine system, despite the considerable efforts made in recent decades to elucidate the modes of coordination in solutions of copper(II) and various histidine-containing peptides of biological importance. For histidylglycine as ligand, only the complexes $[CuL]^+$ and $[CuL_2]$ are taken into consideration in all pH potentiometric studies,[2−5] though most researchers[3−5] also agree on the formation of the dimer $[Cu_2L_2H_{-2}]$. One or more protonated or deprotonated mono or bis complexes have also been assumed in various works. The coordination modes suggested on the basis of pH potentiometric,[2−5] ultraviolet−visible,[3,5] calorimetric,[5] and ESR[6,7] studies display as much divergence. In $[CuL]^+$ and $[CuL_2]$ primarily a histamine-like binding mode is accepted,[2,4,5,7] while the binuclear complex is assumed to be either a dihydroxo-bridged[3] or an imidazolate-bridged[8] species; in other works, loss of the peptide NH proton and the bridging role of the neutral imidazole ring have been suggested.[5,9]

The present paper reports results obtained by two-dimensional simulation of ESR spectra for the copper(II)−L-histidylglycine system. We aimed to identify all detectable species in the pH region 2−12, including possible isomers of the various complexes, and we intend to clarify the competing modes of histamine-like versus dipeptide-like coordination.

Experimental Section

Reagents and Solutions. The ligand L-histidylglycine (denoted by HL in its neutral form) was from Sigma, and other reagents were from Reanal (Hungary); all were of analytical grade and were used without further purification. The copper(II) concentration of the stock solutions was 5 mmol dm^{-3}. The titrations were carried out at ligand:metal concentration ratios of 1:1 and 4:1. We used 0.2 M KCl as background electrolyte. The pH was adjusted with HCl and then with NaOH to an accuracy of 0.01 pH unit, using a Radiometer PHN 240 pH meter equipped with a Radiometer pHC2401 combined glass electrode. The electrode was calibrated with IUPAC standard buffers from Radiometer.

ESR Measurements. The ESR spectra were recorded at 291 ± 0.5 K under an argon atmosphere in a circulating system. All the details are given in an earlier work.[10] The ESR spectra of 5 mM $CuCl_2$ solutions containing 0−0.4 M KCl were also recorded in order to determine the parameters of the aqua complex independently and to check whether the chloride ions of the background electrolyte influence them. (No systematic change in response to variation of the KCl concentration was found.)

Evaluation of Spectra. A series of 36 spectra were evaluated with the 2D_EPR program,[1] modified to take into consideration at most 15 ESR-active species and to fit at most 132 parameters. The spectral analysis was preceded by a correction for the curve of the capillary tube (containing a weak vanadium signal at high field) and the peaks of the manganese(II) standard. The ESR spectra of the various species were described by the parameter g_o, the copper hyperfine coupling constant A_o, the nitrogen superhyperfine coupling constants a_{No}, and the relaxation parameters α, β, and γ, where the different widths of the copper lines can be given as $\sigma_{M_I} = \alpha + \beta M_I + \gamma M_I^2$ (M_I is the magnetic quantum number of copper nuclei). The coupling constants and the field dimension relaxation parameters are given in gauss (G) units throughout the paper; 1 G = 10^{-4} T. The hyperfine coupling constants and the relaxation parameters refer to the isotope ^{63}Cu. The spectra of complexes containing either the isotope ^{63}Cu or ^{65}Cu were calculated and added in the ratio of their natural abundances.

The equilibrium concentrations of the various species were obtained by the solution of mass-balance equations. The optimization of the ESR parameters and the formation constants of the mass-balance equations for the ESR-active complexes was based upon minimization of the overall average square deviation, SSQD, which is the sum of the average square deviations for each spectrum (SQD). The quality of fit for the individual spectra is characterized by a noise-corrected regression parameter R, calculated from SQD.[1] For the comparison of alternative speciation models, the overall regression coefficient for the whole set of spectra, computed from SSQD, was used. The computer program also furnishes the critical value of the difference in overall regression coefficient, which can be regarded as significant for two speciation models. The details of the statistical analysis are to be found in our previous work.[1]

The formation constants of the inactive metal complexes were determined by minimizing the differences in the analytical and the calculated total metal concentrations.

Results and Discussion

Speciation in the Copper(II)−L-Histidylglycine System. The spectra of the copper(II)−L-histidylglycine system in the range pH 2−12 can be best described by assuming one ESR-inactive and 12 active metal complexes. These are the "free" copper(II) ion (aqua complex), the species $[CuLH_2]^{3+}$- and $[CuLH]^{2+}$, the two isomers of $[CuL]^+$, the active binuclear complex $[Cu_2L_2H_{-2}]$ and the inactive one $[Cu_2L_2H_{-3}]^-$, the species $[CuLH_{-2}]^-$ and $[CuL_2H]^+$, the two isomers of $[CuL_2]$, and the complexes $[CuL_2H_{-1}]^-$ and $[CuL_2H_{-2}]^{2-}$. For this model, the overall regression parameter was 0.990538, while the individual R values lay between 0.9823 and 0.9968; for a majority of the spectra, they were between 0.986 and 0.995.

If only the above ESR-active metal complexes are taken into consideration at over pH 11, the total copper(II) concentration calculated from the spectral intensities is considerably decreased relative to the analytical concentration data obtained from the initial concentration and the dilution during the titration (Figure 1). When the speciation model includes the inactive binuclear species $[Cu_2L_2H_{-3}]^-$, however, satisfactory agreement is achieved between the calculated and analytical copper(II) concentrations (Figure 1).

The overall ESR spectroscopic formation constants agree well with the literature pH potentiometric data (Table 1) and

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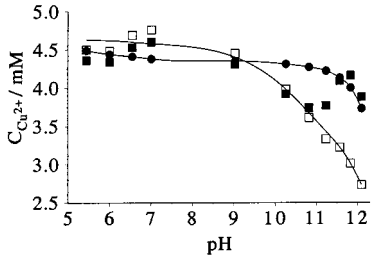

Figure 1. Analytical and calculated total copper(II) concentrations for 1:1 solutions of the copper(II)−L-histidylglycine system, where ● denotes the analytical metal concentration, obtained from the initial concentration and the dilution ratio, changing together with the amount of NaOH solution added; ■ symbolizes the copper(II) concentration calculated from the mass-balance equations by using the ESR spectroscopic formation constants in Table 2; □ denotes the copper(II) concentrations calculated from the previous data, but neglecting the inactive dimer $[Cu_2L_2H_{-3}]^-$.

Table 1. Formation Constants as log β^a for the Copper(II) Complexes of L-Histidylglycine

complex	ref 2	ref 3	ref 4	ref 5	this work[b]
$[CuLH_2]^{3+}$					15.05(7)
$[CuLH]^{2+}$	11.99			12.10	12.17(3)
$[CuL]^+$	8.83	8.02	8.85	8.84	8.91[c]
h isomer					8.50(1)
p isomer					8.70(2)
$[CuLH_{-1}]$	0.76	1.72			
$[Cu_2L_2H_{-2}]$		6.9	8.2	8.22	8.17(3)
$[Cu_2L_2H_{-3}]^-$					−4.30(2)
$[CuLH_{-2}]^-$	−8.74				−9.04(3)
$[CuL_2H]^+$		18.56		20.06	19.98(3)
$[CuL_2]$		14.15	15.06	15.33	14.88[d]
4N isomer					14.67(1)
3N isomer					14.46(2)
$[CuL_2H_{-1}]^-$	5.88	5.66			4.60(4)
$[CuL_2H_{-2}]^{2-}$		−4.1			−7.38(8)

[a] The standard errors of the last digit are given in parentheses. [b] For the proton complexes [LH], $[LH_2]^+$, and $[LH_3]^{2+}$, log β values of 7.58, 13.53, and 16.49, respectively, from ref 4 were used. [c] $\log \beta = \log(\beta_{\mathbf{h}\ \text{isomer}} + \beta_{\mathbf{p}\ \text{isomer}})$. [d] $\log \beta = \log(\beta_{4\text{N}\ \text{isomer}} + \beta_{3\text{N}\ \text{isomer}})$.

particularly with those in refs 4 and 5. The latter publications, however, merely provide data on the complexes formed below pH 8. The previous pH potentiometric literature[2,3] additionally offers information on certain deprotonated species, but the formation constants given there differ to a much greater extent from our data, especially for $[CuL_2H_{-2}]^{2-}$ (Table 1). One possible reason for this is that in refs 2 and 3 a few deprotonated mono and binuclear complexes necessary for a satisfactory description of the ESR spectra were neglected, and this led to the overestimation of the concentrations (and accordingly the formation constants) of the deprotonated bis complexes. We can state quite definitely that it is not necessary to take $[CuL_2H_{-2}]^{2-}$ into consideration for a consistent description of the ESR curves and concentration data up to pH 11. Another source of deviation of the formation constants obtained by pH potentiometry and by our method in the alkaline region may be that the pH potentiometric formation constants are more sensitive than our data to the base error of the electrode above pH 11−11.5. (The two-dimensional ESR method computes the equilibrium concentrations of the metal complexes not from the pH, but from the ESR spectra.) The concentration distribution curves obtained from the ESR spectroscopic formation constants are depicted in Figure 2.

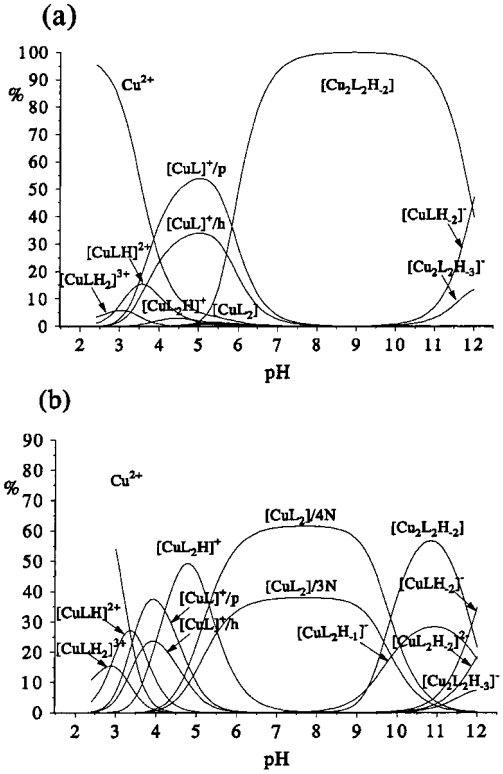

Figure 2. Concentration distribution in the copper(II)−L-histidylglycine system, calculated from the ESR spectroscopic formation constants (a) at equal metal and ligand concentrations; (b) at a 4-fold ligand excess. The isomers of $[CuL]^+$ are distinguished by the mode of coordination (**h** is histamine-like, **p** is peptide-like), and those of $[CuL_2]$ by the number of equatorial N donors. For $[CuL_2]$ in equimolar solution, the sum of the concentrations of the isomers is given.

Confidence of Parameters. The ESR parameters for the various species are listed in Table 2, and the component curves computed from these parameters are illustrated in Figure 3. In general, the standard errors for the various parameters are fairly low, but those for $[CuLH_2]^{3+}$ and $[CuL_2H_{-2}]^{2-}$ are exceptions. The latter both are formed in low concentration and have poorly resolved spectra (Figures 2 and 3). Moreover, the aqua complex, for which the copper hf splitting is not resolved, is also present in a relatively large concentration when $[CuLH_2]^{3+}$ is formed, while $[CuL_2H_{-2}]^{2-}$ is represented in only a small number of spectra. All these factors together can explain the reduced level of confidence in their parameters. This prohibited the investigation of possible isomerism for the latter species.

Identification of Equatorial Donor Groups in Various Species. Previous experiments on several copper(II)−amino acid and −peptide systems[1,10−12] demonstrated that ESR parameters are a rich source of information regarding equatorial coordination. (1) The g_o values are determined by the equatorial donor groups; the axial ligands have only small and indirect effects on them, since the ground state of the copper(II) ion is $d_{x^2-y^2}$. (2) As the equatorial water molecules are displaced by the donor atoms of the ligand,

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Table 2. ESR Parameters for the Various Complexes in the Copper(II)−L-Histidylglycine System[a]

complex		g_o	A_o	a_{No}	α	β	γ
Cu^{2+}		2.1956(3)	34.6(4)		49.5(8)	−1.8(3)	0.6(2)
$[CuLH_2]^{3+}$		2.172(3)	40.(5)	7.0(8)	52.8(9)	−5.0(3)	1.0(2)
$[CuLH]^{2+}$		2.1500(8)	64.3(7)	9.1(7)	38.1(1)	−13.4(1)	2.6(1)
				9.1(7)			
$[CuL]^{+}$	**h** isomer	2.1491(3)	61.1(2)	12.7(3)	26.5(1)	−11.1(1)	0.4(1)
				9.8(4)			
	p isomer	2.1323(9)	54.5(8)	14.0(2)	41.0(1)	−5.5(1)	0.6(1)
				13.0(1)			
$[Cu_2L_2H_{-2}]$		2.1041(3)	25.(5)		55.2(4)	−7.4(7)	3.0(5)
$[CuLH_{-2}]^{-}$		2.1160(1)	36.4(1)	12.4(3)	19.2(1)	−9.5(1)	2.0(1)
				12.4(3)			
$[CuL_2H]^{+}$		2.1214(9)	66.1(5)	13.7(9)	44.7(8)	−26.2(2)	6.6(2)
				7.(2)			
				6.(2)			
$[CuL_2]$	4N isomer	2.1068(2)	60.7(2)	9.6(6)	45.7(2)	−19.7(1)	0.1(1)
				9.6(6)			
				9.6(6)			
				7.8(8)			
	3N isomer	2.1228(3)	64.9(3)	9.2(6)	31.7(1)	−17.2(1)	5.3(1)
				7.2(8)			
				7.0(8)			
$[CuL_2H_{-1}]^{-}$		2.1123(7)	41.2(8)	16.0(1)	21.0(1)	−9.2(1)	1.7(1)
				13.0(2)			
				11.0(1)			
$[CuL_2H_{-2}]^{2-}$		2.071(4)	46.0(3)	15.0(6)	35.0(7)	−8.0(4)	0.0(1)
				15.0(6)			
				8.0(6)			
				8.0(6)			

[a] The hyperfine coupling constants and the relaxation parameters given in G refer to the isotope ^{63}Cu; the standard errors of the last digit are given in parentheses.

g_o decreases and A_o increases. These changes follow the shift of the visible absorption band; accordingly, the sequence of increasing effect[13] is as follows: carboxylate O < imidazole < amino < deprotonated peptide N. OH^- and peptide O ligands are almost as weak as water.[13] The coordination of a deprotonated pyrrolic N, similarly to that of a deprotonated peptide N, induces a considerable decrease in g_o.[10] (3) The unexpectedly low value of A_o sometimes affords additional information on the lower symmetry of the ligand field.[1,11,12,14,15] (The theoretical background of these statements and the relevant experimental evidence are discussed in detail in our previous papers[1,10] and the references therein.)

With regard to the above points, to decide which of the competing histamine-like and dipeptide-like binding modes occurs in our species, we can obtain reliable information by comparing the ESR parameters of our complexes with those of the complexes of L-histidine and histamine,[10] and glycylglycine.[11] The g_o and A_o values obtained in the above systems, together with the natures of the equatorial donor atoms, are presented in Table 3.

Coordination Modes in Mono Complexes and Binuclear Species. In $[CuLH_2]^{3+}$, either the carboxylate group can bind to the metal ion, both N donors remaining protonated, or one of the N donors is coordinated, while the other N and the carboxylic O remain in the protonated state. The g_o value (Table 2) indicates the latter case: it is significantly lower than g_o for the complex $[CuLH_2]^{3+}$ of L-histidine with carboxylate coordination, and nearly equal to g_o for the histamine complex $[CuLH]^{3+}$ (Table 3). Accordingly, as for the latter species, we suggest monodentate imidazole binding, with the amino and carboxylate groups protonated (Figure 4). The difference in the coordination of histidine and histidylglycine corresponds to the different acidities of their carboxylic groups: in a majority of the free histidylglycine molecules, the carboxylic group is protonated in the pH range where $[CuLH_2]^{3+}$ is formed, in contrast with histidine; the corresponding deprotonation pK values[4] are 2.96 and 1.50, respectively.

The value of g_o for $[CuLH]^{2+}$ (Table 2) is close to those for the complexes [CuL] of L-histidinec and histamine (Table 3), in which bidentate equatorial coordination by the amino and imidazole N atoms occurs. A similar binding mode is most probable for the histidylglycine complex, too (Figure 4). The visible spectroscopic and calorimetric data led Daniele et al.[5] to suggest this histamine-like coordination and a protonated carboxylic group for $[CuLH]^{2+}$.

Proton loss from the former species yields two different modes of coordination: one of the isomers of $[CuL]^+$ can be described by almost the same parameters as those for $[CuLH]^{2+}$, which points to its histamine-like equatorial coordination; accordingly, it is symbolized by **h** (Figure 4). In this species, the carboxylate group cannot take part in the equatorial coordination for steric reasons. We expect, therefore, that the deprotonation pK for $[CuLH]^{2+}$ agrees with the value of 2.96 for the carboxylic group of the free ligand[4] (or, in the event of a possible weak axial coordination, it will be slightly less). In contrast, for the deprotonation process leading to formation of the **h** isomer, the pK is 0.71 log unit higher (3.67 from the data in Table 1). This can be explained by the stronger inductive effect of the positively charged, protonated amino and imidazole N groups in the

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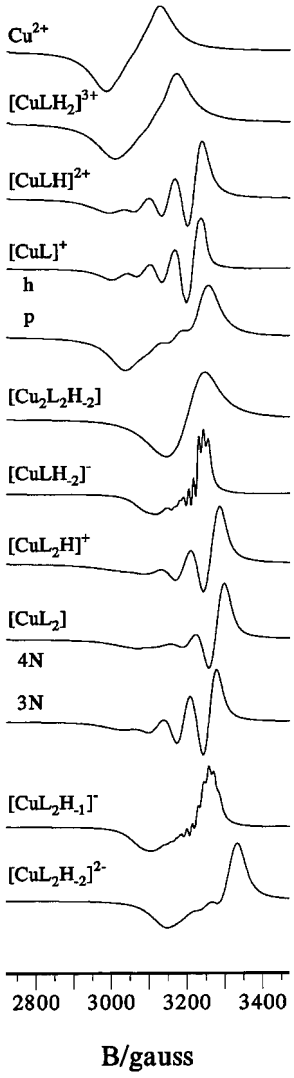

Figure 3. Calculated ESR spectra for all active complexes assigned in the copper(II)−L-histidylglycine system, by using the data in Table 2 at a frequency of 9.4 GHz.

free ligand, which promotes the loss of proton from the carboxylic group, while in the complex the coordination of the N donors significantly reduces this effect. For the other isomer of $[CuL]^+$, denoted by **p**, g_o is significantly lower, indicating a considerably stronger ligand field. Here, metal ion-induced deprotonation of the peptide NH most probably takes place, and the amino and deprotonated peptide N atoms and the carboxylate O donor are coordinated in equatorial positions, as in the complexes $[CuLH_{-1}]$ of simple dipeptides (Figure 4); the imidazole ring remains protonated.

The coordination proposed above for $[CuL]^+$ is in accord with the conclusion of Daniele et al.,[5] based upon visible spectroscopic data and the comparison of formation entropies: this species exhibits both histamine-like coordination and weak (partial) charge neutralization of the carboxylate group by the metal ion. However, Daniele et al.[5] assumed that these interactions act in the same molecules, whereas we interpret our ESR findings in terms of an isomeric equilibrium: there are complexes where the carboxylate−copper(II) bond is promoted by simultaneous coordination of the amino and deprotonated peptide N donors (**p** isomer), but in other molecules involving histamine-like binding this interaction is negligible (**h** isomer).

At equal metal ion and ligand concentrations, in neutral or slightly alkaline solution, the ESR-active dimer $[Cu_2L_2H_{-2}]$ predominates. Its broad, nonresolved spectrum indicates the dipole−dipole interaction between the copper(II) centers. Three possible structures were earlier proposed for this complex: (1) two monomer units involving histamine-like coordination are connected by two OH^- ions;[3] (2) in the monomer units the coordination also takes place through the amino and imidazole N atoms, while the pyrrolic N atoms lose protons and mutually bind to the neighboring metal ions;[8] and (3) in the monomer units, the peptide NH undergoes deprotonation, and the coordination is reminiscent of that in the complexes $[CuLH_{-1}]$ of simple dipeptides; these units are then connected by the imidazole N atoms which mutually occupy the fourth equatorial site of the other metal ion.[5,9]

The first suggested mode of coordination can be rejected for two reasons: first, the fact of ESR activity suggests that the interaction between the paramagnetic centers is not too strong, while hydroxo bridges generally mediate strong interactions leading to the spin-pairing of copper(II) ions;[8] second, if a dihydroxo-bridged, otherwise histamine-like structure were formed, in the event of ESR activity a much higher g_o (nearly identical to that for the **h** isomer of $[CuL]^+$) would be expected since OH^- is a weak donor. The thermodynamic data[5] likewise provide evidence against the first structure.

The metal ion-induced deprotonation of the ligands suggests strong equatorial connections between the metal ions and the deprotonated N donors. The low g_o corresponds to three equatorial N donors, in accordance with either the second or the third structure. On the basis of g_o alone, however, we cannot decide which of them is formed. The second structure can be rejected for the following reason. Molecular models show that the histamine-like equatorial coordination to one copper(II) ion and simultaneous equatorial ligation of the other N of the imidazolate ring to the other metal ion, and vice versa, are sterically hindered. At most a weak axial bridging interaction with a considerably distorted bond angle would result, which would not support a strong 3N equatorial interaction and a low g_o. Accordingly, loss of proton from the peptide NH rather than from the imidazole ring is likely in the active binuclear complex: we suggest the third, dipeptide-like coordination, together with imidazole bridges (Figure 4).

For $[CuLH_{-2}]^-$, g_o is similar, while A_o is much lower than for the histamine and histidine complexes $[CuLH_{-1}]$ and $[CuLH_{-2}]^-$ involving imidazolate coordination, where the pyrrolic nitrogen (and an equatorial water molecule in the second species) is deprotonated (Table 3). At the same time, all the ESR parameters of $[CuLH_{-2}]^-$, including the superhyperfine coupling constants of two equivalent N atoms (Table 2), are closely identical to those of the mixed hydroxo complex $[CuLH_{-1}(OH)]^-$ of glycylglycine,[11] indicating dipeptide-like coordination again: proton loss from the

Table 3. ESR Parameters[a] and Nature of Equatorial Coordination for the Copper(II)−L-Histidine, Copper(II)−Histamine, and Copper(II)−Glycylglycine Complexes[b]

	ligand								
	L-histidine[c]			histamine[c]			glycylglycine[d]		
complex	g_o	A_o	coordination[e]	g_o	A_o	coordination[e]	g_o	A_o	coordination[e]
$CuLH_2$	2.189	37	carb						
CuLH	2.154	57	am, carb	2.175	35	im			
CuL	2.146	60	am, im	2.144	63	am, im	2.157	48	am, pept-O
$CuLH_{-1}$	2.119	66	am, im^-				2.121	67	am, pept-N^-, carb
$CuLH_{-2}$	2.119	76	am, im^- OH^-	2.116	78	am, im^- OH^-	2.117	37	am, pept-N^-, carb OH^-
CuL_2H	2.119	72	am, im am, carb	2.132	62	am, im im			
CuL_2									
4N isomer	2.114	63	am, im am, im	2.101	70	am, im am, im			
3N isomer	2.122	67	am, im am, carb (im ax)	2.115	74	am, im am (im ax)			
CuL_2H_{-1}									
3N isomer							2.104	45	am, pept-N^-, carb am (pept-O ax)
2N isomer							2.116	53	am, pept-N^-, carb pept-O (am ax)

[a] A_o refers to the isotope ^{63}Cu; it is given in G. [b] The neutral L-histidine and glycylglycine are denoted by HL, and histamine is denoted by L. As the charges of the analogous complexes of different ligands are different, they are omitted. [c] Data from ref 10. [d] Data from ref 12. [e] Abbreviations: carb: carboxylate-O, am: amino-N, im: imidazole-N, pept: peptide, ax: axial position.

peptide NH and an equatorial water molecule in the histidylglycine complex (Figure 4). Another analogy with the simple dipeptides is that (together with the above mixed hydroxo complex) a small amount of the diamagnetic binuclear species $[Cu_2L_2H_{-3}]^-$ is formed. Its ESR inactivity suggests that the $[CuLH_{-1}]$ units are connected by a hydroxide ion.[8,12] In the case of histidylglycine, formation of the above (monomeric and dimeric) hydroxo complexes requires the breakdown of the imidazole bridges of the active dimer, which can explain why the formation is shifted upward by about 2−3 pH units as compared to the simple dipeptides.

Coordination in Bis Complexes. For $[CuL_2H]^+$, the coordination of L may be either histamine-like or dipeptide-like, as in the isomers of $[CuL]^+$. LH can be bound by either the imidazole or the amino N atoms (together with the peptide O in the latter case). Despite this variety of possible structures, we were able to identify only one kind of coordination for this species. The g_o value alone does not provide decisive evidence regarding its structure. If we assume histamine-like equatorial coordination for L, comparison with the **h** isomer of $[CuL]^+$ reveals a decrease of 0.028 in g_o (Table 2) upon coordination of L. This shift in g_o seems to be too large for either monodentate imidazole coordination of the second ligand (comparing the species $[CuL]^{2+}$ and $[CuL_2H]^{3+}$ of histamine) or its binding by the amino N and peptide O donors. In general, the equatorial binding of an amino N reduces g_o to such an extent only if it is accompanied by the simultaneous equatorial ligation of a donor stronger than the peptide O, e.g., a carboxylate O (see, for instance, the differences in g_o between $[CuL]^+$ and $[CuL_2H]^+$ or the 3N isomer of $[CuL_2]$ for L-histidine, Table 3). However, the amino-N + carboxylate-O coordination, without participation of the peptide group, is not favored for our LH, since an eight-membered chelate ring would form. In other words, the explanation of g_o on the assumption that L is bound in a histamine-like manner seems less reasonable. Amino-N,imidazole-N + amino-N,peptide-O equatorial coordination, however, cannot definitely be excluded on the basis of the shift in g_o: there is an example (the 3N isomer of the histamine $[CuL_2]^{2+}$ complex) where equatorial ligation of a single amino N to the complex $[CuL]^{2+}$ decreases g_o by 0.029. In any event, if the coordination of L is still histamine-like, LH is connected to the metal ion by the amino group (Figure 5, structure **a**). If, in turn, we assume a peptide-like binding mode for L and compare $[CuL_2H]^+$ with the **p** isomer of $[CuL]^+$, we obtain a g_o shift of 0.011. This corresponds to coordination of the imidazole rather than the amino N of LH: the bonding of an amino group to the fourth equatorial site of $[CuLH_{-1}]$ for the simple dipeptides[11,12] decreases g_o by 0.017−0.020, while monodentate imidazole coordination to the species $[CuL]^{2+}$ of histamine induces a shift of 0.012 (Table 3). The corresponding structure **b** is depicted in Figure 5.

Although the ESR data do not provide sufficient evidence, the literature thermodynamic data[5] support structure **b** for $[CuL_2H]^+$. From the formation entropy data, it was concluded[5] that a carboxylate group is neutralized in this complex. This can be achieved either by protonation or by coordination to the metal ion. The former possibility was put forward in ref 5, together with bis-histamine-like coordination. The deprotonation pK of 5.1 of this complex (from the data in Table 1), however, would be unreasonably high to allow assignment to an unbound carboxylic group in the complex. (For the free ligand, the corresponding pK[4] is only 2.96.) It is much more probable that the carboxylate group is neutralized by metal ion coordination, in accordance with structure **b**.

In $[CuL_2]$, LH_{-1} + LH coordination would require an unreasonably high basicity of the unbound N-containing donor group of LH in the complex (with a deprotonation pK of 10.51 from the data in Table 1) relative to that for the

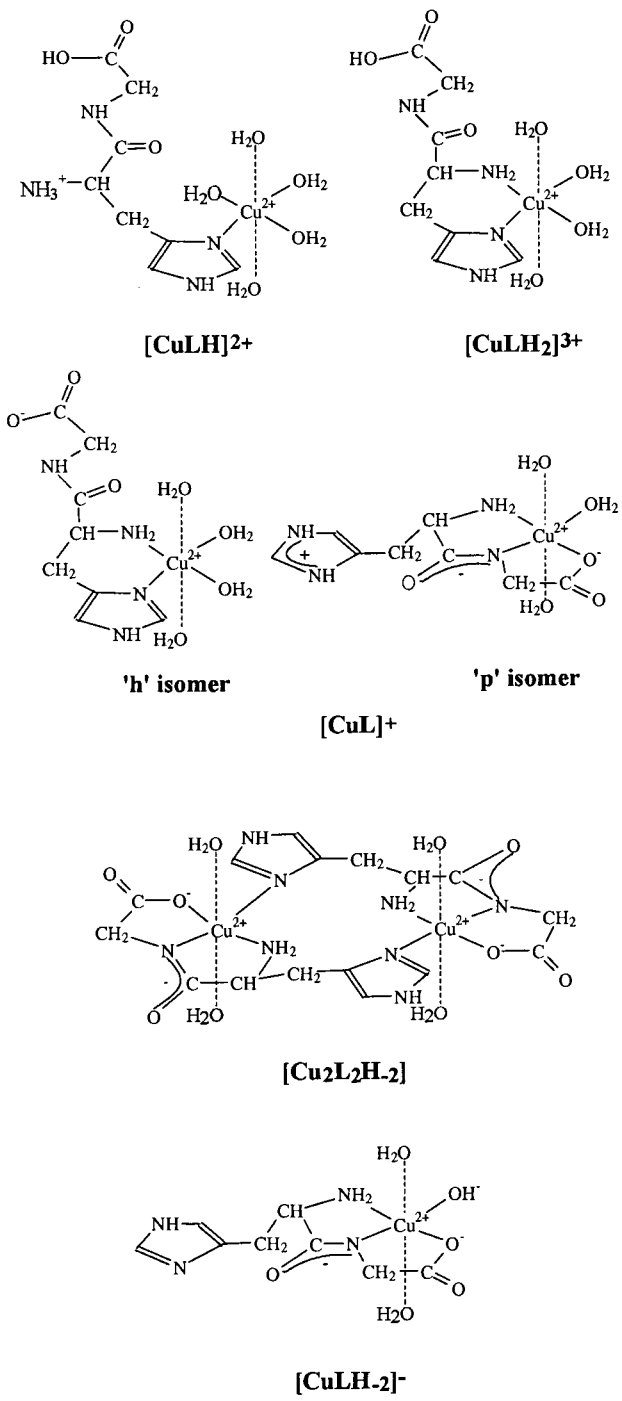

Figure 4. Coordination modes proposed for the various ESR-active mono complexes and dimer of L-histidylglycine and copper(II).

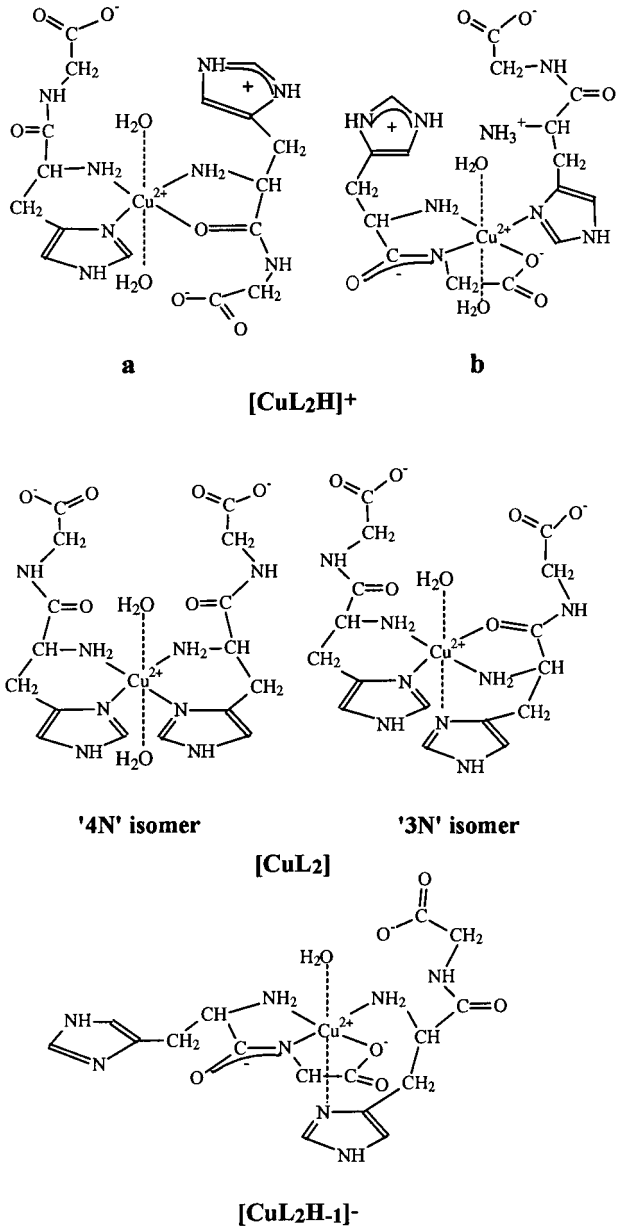

Figure 5. Coordination modes proposed for the various bis complexes of L-histidylglycine and copper(II).

free ligand (deprotonation pK = 5.95 for the imidazole and 7.58 for the amino group[4]). Accordingly, both ligands are most probably in L form, and bis-histamine-like coordination by the amino and imidazole groups occurs. Simultaneously, we have found two different arrangements of donor groups (see above). Here, two kinds of isomerism may occur. In the event of diequatorial coordination of both ligands, the donors of the same kind may occupy *cis* or *trans* positions. The other possibility is that either both ligands bind in equatorial positions or the coordination of the first is diequatorial and that of the second histidylglycine molecule is equatorial-axial. In the former case, nearly equal g_o values for the isomers would be expected, their equatorial donor atom sets being the same. For the histidylglycine complex, however, the considerable difference in g_o (Table 2) suggests rather the second kind of isomeric equilibrium, which was concluded for the analogous L-histidine and histamine complexes,[10] too (Table 3). For the major, 4N isomer with lower g_o and A_o, we suggest the equatorial coordination of all four N donors and (according to the low A_o) the lower symmetry *cis* arrangement of the donor atoms of the same kind (Figure 5). For the minor, 3N isomer, g_o is higher by 0.013, which corresponds to the lack of an imidazole N from the equatorial coordination: thus, this group is likely to bind in an axial position (Figure 5).

For $[CuL_2H_{-1}]^-$, the question is whether LH_{-1} is bound only by the amino and deprotonated peptide N atoms, when

L displaces its carboxylate donor and also occupies two equatorial sites, or the moiety LH_{-1} preserves the usual tridentate binding mode, leaving only one free equatorial position for the other histidylglycine molecule. In the first case, in comparison with the **p** isomer of [CuL], the lack of a carboxylate group would increase g_o by about 0.005−0.01, while the histamine-like diequatorial ligation of L would cause a decrease of about 0.040 (see the differences between [CuL] and the 4N isomers of the species $[CuL_2]$ of histamine, L-histidine, and L-histidylglycine in Tables 2 and 3). When both effects are taken into consideration, a g_o value lower than 2.100 would be expected for the above-mentioned 4N coordination. Thus, the g_o value of 2.114 (Table 2) rather supports tridentate coordination of the deprotonated ligand and the presence of only three equatorial N donors. The deviation from the g_o for the **p** isomer is 0.018, the same as the change accompanying the connection of an amino N at the fourth equatorial site in the 3N isomer of $[CuL_2H_{-1}]^-$ for the dipeptides with noncoordinating or weakly coordinating side-chains (Table 2, ref 12). A similar equatorial coordination is therefore also proposed for the histidylglycine complex (Figure 5), where the imidazole N of L occupies an axial site. A further analogy lends support to this structure: the very low value of A_o, which is indicative of significant rhombic distortion. Such a distortion is observed in a number of copper(II) complexes where amino groups of two peptide or amino acid ligands occupy neighboring equatorial sites (Table 2 and refs 1, 11, 12, 14, 15).

Conclusions

Two-dimensional ESR spectroscopic analysis has shown that, in the presence of copper(II) ion, the competition between the histamine-like N-terminal part and the dipeptide backbone of the ligand L-histidylglycine results in a variety of coordination modes, and the backbone donors are of much higher importance than assumed previously. In strongly acidic media, the side-chain plays a decisive role by participating in monodentate imidazole binding and then histamine-like equatorial ligation through the amino and imidazole donors ($[CuLH_2]^{3+}$ and $[CuLH]^{2+}$, respectively). In moderately acidic solution, deprotonation of the peptide group also occurs, with somewhat higher probability than that for histamine-like coordination (isomerism for $[CuL]^+$). At equal metal and ligand concentrations, in the neutral and alkaline region up to pH 12, peptide-like tridentate equatorial coordination by the backbone donors takes over the leading role from the histamine-like binding mode. Here, the side-chain is only a bridging group, though a strong one (active dimer $[Cu_2L_2H_{-2}]$), and even this role ceases in strongly alkaline solution (hydroxo complexes $[Cu_2L_2H_{-3}]^-$ and $[CuLH_{-2}]^-$). At a ligand excess, in nearly neutral media, the N-terminal part and histamine-like coordination achieves an advantage over the backbone: the diequatorial coordination of the first L is accompanied by diequatorial or equatorial-axial ligation of the second (isomers of $[CuL_2]$). At above pH 9, the backbone again predominates in the deprotonated ligands of the above-mentioned mono complexes and binuclear species, and $[CuL_2H_{-1}]^-$). In the latter case, histamine-like ligation of the second ligand also takes place.

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Supporting Information Available: Listing of overall regression coefficients for the best equilibrium model and for reduced ones; figures that illustrate the impairment of spectral fit at various ligand concentrations and pH, when neglecting one or other complex implicated in the best model. This material is available free of charge via the Internet at http://pubs.acs.org.

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