ESR STUDY OF THE STRUCTURE AND BONDING PARAMETERS IN BINARY COPPER(II) COMPLEXES OF SOME α-AMINO ACIDS AND DIPEPTIDES*

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Binary 1 : 1 copper(II)-amino acid and copper(II)-dipeptide complexes, [CuL] and $[CuL']^+$ ($H_2L = {}^+H_3N-CHR-CO-NH-CHR'-COO^-$, $HL' = {}^+H_3N-CHR-COO^-$), have been investigated in aqueous solution by means of ESR and electron absorption spectroscopy. Molecular orbital coefficients characteristic of the metal–ligand bonds have been derived for an effective D_{4h} local symmetry. It is suggested that at the pH near to the physiological conditions both histidine and tryptophan coordinate as a tridentate ligand via O(carboxyl), N(heterocyclic ring) and N(amino) atoms. ESR investigation at room temperature and in frozen aqueous solutions, and visible spectral evidence suggest histamine-like coordination of histidine and tryptamine-like coordination of tryptophan in the quatorial plane of the binary complexes. Since proline contains the imino group, there is no ionizable amide-NH-proton when it is inserted in a peptide chain, hence the proline-nitrogen is unable to bind metal ions in peptides.

The interactions between metals and amino acids and peptides have become of considerable interest as coordination phenomena, as models for metal–protein reactions, and as models for biological systems in which the properties of proteins are modified by the fact that metal atoms are attached to them. Extensive investigations[2,3] have shown that dipeptides without any function group in the backbone form a 1 : 1 complex with copper(II) with many complex species contributing to this system. Unfortunately a detailed description requires the accumulation of massive equilibrium data, and our knowledge of other metal–peptide systems has often based on much scantier experimental evidence. Predominant species presented in 1 : 1 Cu(II)-dipeptide solutions at pH near to 7 are[2] depicted in Fig. 1. Dipeptides that form chelates to Cu(II) through amino nitrogen, deprotonated amide nitrogen, and carboxylate oxygen donors (*III*) prevail[2,3]. Since ESR measurements on complexes in solution can provide direct evidence for the number of N atoms coordinated to Cu(II), ESR spectroscopy has been widely used to study complexes formed in solu-

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tions between the copper(II) ion and various ligands[4], including α-amino acids[5-7] or dipeptides[8]. However, in spite of increasing information provided even by X-ray analysis[9,10], there is still uncertainty as to which of the coordination sites of potentially tridentate ligands are involved in the binding of metal ions, particularly in solutions.

The present work was undertaken to provide ESR information about the structure of some binary amino acid-copper(II) and dipeptide-copper(II) complexes. Two series of compounds have been examined in an attempt to interpret their structure in view of the different donor ability of the ligands. The ESR parameters have been used to compute molecular orbital coefficients in the knowledge of the energies of the ligand field electronic transitions. The coordination modes and their influence on the covalent character of the metal–ligand bonds have been studied.

EXPERIMENTAL

L-Amino acids of analytical grade from Fluka were used without further purification for preparation of the solutions. Dipeptides glycylglycine (GlyGly), glycyl-L-leucine (GlyLeu), and glycyl--L-tryptophan (GlyTry) were of Reanal and 99·7% chromatographic grade; glycyl-L-proline (GlyPro) was kindly supplied free of charge from Serva Feinbiochemica (Heidelberg). Histamine (Hm) and ethylenediamine (en) were of Merck. All other chemicals were of the highest grade available. The spectra of the binary species were obtained on aqueous solutions containing copper(II) and the amino acid or dipeptide, respectively, in the ratio 1 : 1 (concentration: $5 . 10^{-3}$ mol dm^{-3}) at pH 6·5—7·0. No buffers were used, in order to avoid interaction of Cu(II) with other ligands. Solutions for optical absorption measurements were prepared by slowly adding 2M-NaOH to aqueous solutions initially at pH 3. A pH-meter PHM-4 Radiometer with glass and SCF electrodes was used.

Electron spin resonance spectra were recorded on an ERS 230 (Academy of Sciences GDR, ZWG Berlin) instrument. First derivative ESR X-band (ca. 9·4 GHz) spectra were obtained; microwave power 5 mW, modulation amplitude 0·5 mT. Both solution and glass spectra were obtained using DMSO–water mixtures (1 : 4 and 1 : 1 (v/v) ratio, respectively) at 295 or 103 K, respectively. The g values were calibrated against diphenylpicrylhydrazyl (DPPH). For solution

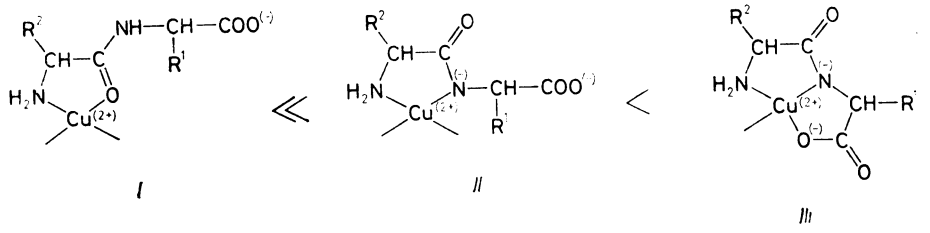

FIG. 1
Predominant species present in 1 : 1 Cu(II) dipeptide solutions

spectra measurements flat cells were used. Electronic spectra were measured with a Specord M 40 spectrophotometer (Carl Zeiss), glass cells being used.

CALCULATIONS

ESR results were evaluated by assuming an effective D_{4h} local symmetry of the complexes. In this case, the symmetry-adapted antibonding molecular orbitals of the Cu(II) ion can be written[11] as shown in Eqs (*1*) to (*4*):

$$\psi^*_{B_{1g}} = \alpha d_{x^2-y^2} - \alpha' \varphi_L(x^2 - y^2) \tag{1}$$

$$\psi^*_{B_{2g}} = \beta_1 d_{xy} - \beta'_1 \varphi_L(xy) \tag{2}$$

$$\psi^*_{A_{1g}} = \alpha_1 d_{z^2} - \alpha'_1 \varphi_L(z^2) \tag{3}$$

$$\psi^*_{E_{1g}} = \beta d_{xz} - \beta' \varphi_L(xz) \tag{4a}$$

$$\psi^*_{E_{1g}} = \beta d_{yz} - \beta' \varphi_L(yz) , \tag{4b}$$

where α, β_1, and β are the coefficients which express the covalent character of the σ-bonding in-plane and out-of-plane π bonding, respectively and the other symbols have their usual significance. These coefficients can be obtained from the relations which connect them with the spin-Hamiltonian parameters for the axial symmetry[11-13]. The α'^2, out-of-plane σ-bond strength, was calculated using the relationship (*5*)

$$\alpha^2 + \alpha'^2 - 2\alpha\alpha' S = 1 , \tag{5}$$

where S is the overlap integral which is taken as 0·093 (ref.[11]). The experimental values of isotropic parameters, g_0 and A_0, were derived from the spectra taken at room temperature. The $g_\parallel$, $A_\parallel$, and $g_\perp$ values were estimated graphically from the glass spectra. In the description of the ESR data obtained for the cupric ion, use was made of the theory of molecular orbitals for copper complexes[11,12]. The Fermi hyperfine contact term K is calculated from Eq. (*6*)

$$K = -A_0 + P(g_0 - g_e) , \tag{6}$$

where $g_e = 2{\cdot}0023$ and $P = 0{\cdot}036\ \mathrm{cm}^{-1}$ for Cu(II) ion[13].

The MO coefficient ε' which characterizes the covalent character of the 4*s* σ-bond in the case of an effective D_{4h} symmetry, can be calculated from the Fermi hyperfine contact term[13].

RESULTS AND DISCUSSION

At room temperature the species presented in aqueous solution gave rise the isotropic ESR spectra consist of four $(2I + 1)$ absorption peaks arising from coupling of the electron spin dipole $(S = 1/2)$ with the copper nuclear spin dipole $(I = 3/2)$. At 103 K typical glassy spectra were obtained, all characterized by three well-resolved hyperfine lines on $g_{\parallel}$ and the complex shape of the perpendicular region. An "overshoot" signal at g c. 2·00, as is often the case in copper(II) complexes[4], accounts for intermediate orientations relative to the external magnetic field. All the spectra are characterized by both $g_{\parallel}$ and $g_{\perp}$ values higher than 2·040, corresponding to a ground state configuration with the unpaired electron in the $d_{x^2-y^2}$ orbital. Both the spin-Hamiltonian parameters listed in Table I, such as the $A_{\parallel}$ values, and the shape of the perpendicular region in the glassy spectra are typical of tetragonal Cu(II) complexes with weak axial perturbation and strong in-plane ligands[6].

Amino Acid Complexes

The normal mode of amino acid coordination is through the amino and carboxylate groups. The lower the pK_a, the greater is the availability of the donor atom for the

TABLE I
ESR and visible absorption parameters for binary complexes[a]

Complex	$g_{\parallel}$	$g_{\perp}$	$A_{\parallel}$	g_0	A_0	$\lambda_{max}(\varepsilon_{max})^b$	$n_{N(eq)}$
$[Cu(H_2O)_6]^{2+}$	2·421	2·082	119				0
$[Cu(Gly)]^+$	2·329	2·079	157	2·167	57	681 (35)	1
$[Cu(Gly)_2]$				2·126[c]	67[c]	619	2
$[Cu(Pro)]^+$	2·300	2·077	176	2·165	59	677 (36)	1
$[Cu(Pro)_2]$				2·118[c]	70[c]	604	2
$[Cu(Phe)]^+$	2·263	2·070	180	2·164	56	683 (41)	1
$[Cu(Phe)_2]$				2·123[c]	68[c]		2
$[Cu(Try)]^+$	2·262	2·066	176	2·123	63	644 (36)	2
$[Cu(His)]^+$	2·294	2·069	169	2·157	61	661 (45)	2
$[Cu(His)_2]$	2·238[d]	2·058[d]	183[d]	2·119[c]	71[c]	642	3
$[Cu(Hm)]^{2+}$	2·292	2·078	167	2·156	67	668 (32)	2
$[Cu(en)]^{2+}$	2·292[e]	2·062[e]	168[e]	2·132[c]		641 (27)	2
[Cu(GlyGly)]	2·239	2·044	173	2·130	69	655 (53)	2
$[Cu(GlyPro)]^+$	2·289	2·075	153	2·169	52	717 (38)	1
[Cu(GlyLeu)]	2·230	2·047	164	2·119	74	651 (57)	2
[Cu(GlyTry)]	2·233	2·042	166	2·116	72	642 (71)	2

[a] A values in units of 10^{-4} cm^{-1}; [b] λ in nm, ε in l mol^{-1} cm^{-1}; [c] data from ref.[5]; [d] values from ref.[6]; [e] values from ref.[7].

formation of a metal–ligand bond.* According to this criterion, the order of metal-binding tendencies will be carboxyl > imidazole > amino ($pK(COOH) \sim 1{\cdot}8$, $pK(ImH_2) \sim 6{\cdot}5$, $pK(NH_3) \sim 9{\cdot}0$)[2]. Calculations[5] of the percentage distribution of copper amongst the various complexes (Cu^{2+}, $[Cu_2(OH)_2]^{2+}$, $[CuL']^+$, $[CuL'_2]$) for the Cu(II)-amino acid (1 : 1) system show, that in the pH range 5–7, the copper is present entirely as the mono-complex $[CuL']^+$. The spectrum in Fig. 2, nominally from $[CuL']^+$ (L' = Gly), shows a high-field component arising from the presence of $[CuL'_2]$; also the shape of the low-field peak is affected by the presence of some $Cu^{2+}(aq)$, which in solution at room temperature gives a single broad peak. The character of the spectra is the same for $[CuL']^+$ complexes, where L' = Pro and Phe, however the latter shows only a small high-field component. For $[CuL'_2]$ complexes of those, values of g_0 and A_0 were reported previously[5] (see Table I).

The ESR spectra of the other $[CuL']^+$ complexes exclude formation of the $[CuL'_2]$ species at the pH near to the physiological conditions and Cu(II) : L' ratio of 1 : 1.

For ligands other than Hm and en the visible absorption spectra are consistent with two nitrogen atoms coordinated in $[CuL]$ or $[CuL']^+$ (L' = Try, His), where $\lambda_{max} < 660$ nm, and one nitrogen coordinated in $[CuL]^+$ or $[CuL']^+$, where L' = = Gly, Pro, Phe ($\lambda_{max} > 675$ nm). Generally, as the pH is raised from acid values, more nitrogen atoms coordinate to the metal ion (cf. Fig. 1) with a concomitant shift of the optical absorption to higher energies.

The main question under debate is whether histidine coordinates in a histamine-like fashion (with the amino and the imidazole N atoms in the equatorial positions), or one is bound to the Cu(II) in a glycine-like manner.

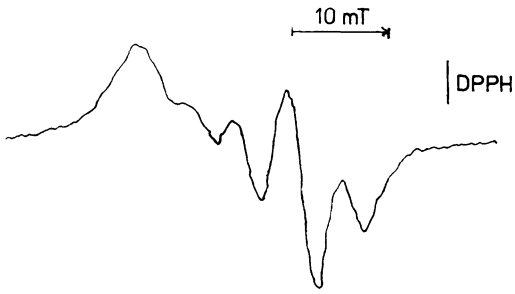

FIG. 2
First-derivative X-band ESR spectrum of the $[Cu(Gly)]^+$ comples in 20% (v/v) DMSO–water at room temperature

* It is risky to use this criterion alone, because the order of the pK values may not be the same as that of the enthalpy changes accompanying complex formation, which provide a measure of the relative thermodynamic stabilities of the metal–ligand bonds. Finally, bonds with low enthalpies of formation may nevertheless be stabilized by favourable entropy effects. However, for our purpose pK_a criterion is acceptable.

In interpreting the ESR spectra for 1 : 1 Cu(II)-histidine and Cu(II)-tryptophan complexes it is necessary to note the distinctive spectroscopic parameters for α-amino acid complexes, $[Cu(en)]^{2+}$ and $[Cu(Hm)]^{2+}$ complexes (Table I). The observed values of g_0 and A_0 in tetragonal symmetry range from about 2·165 and $57 . 10^{-4}$ cm^{-1}. respectively, in complex with one N atom in the equatorial plane to less than 2·135 and greater than $60 . 10^{-4} cm^{-1}$, respectively, in complexes in which Cu(II) is surrounded by two N atoms. Similar variations exist in $g_{\parallel}$ and $g_{\perp}$. Thus it is evident the nephelauxetic effect does reduce the g factor, e.g., the largest g factors are obtained with oxygen as ligand(s), smaller ones result from coordination to nitrogen(s).

The present work provides the following information on the coordination of histidine and tryptophan to Cu(II): (i) The complex $[Cu(His)]^{+}$ certainly consists of a tridentate His, which equatorially coordinates through amino- and imidazole nitrogen atoms in a square-planar arrangement and carboxylate oxygen in apical position, because, although the value of A_0 of $53 . 10^{-4} cm^{-1}$ is only slightly smaller than those for other α-amino acidate complexes, the other ESR and visible absorption parameters are very similar to those for the $[Cu(Hm)]^{2+}$ complex in which two N atoms are equatorially coordinated to Cu(II). (ii) The complex $[Cu(Try)]^{+}$ has two N atoms equatorially coordinated to the Cu(II) and O(carboxylate) is coordinated axially by the same manner as in the $[Cu(His)]^{+}$ complex. Therefore, tryptophan also acts as a tridentate ligand because the value of g_0 is closely related to those for dipeptide complexes with two N atoms coordinated in the equatorial plane. These complexes also provide similar absorption spectra (Table I).

Dipeptide Complexes

From previous study[2] it is obvious that predominant species present in 1 : 1 Cu(II)--dipeptide solutions are [CuL] species where a dipeptide anion (L) coordinates through amino nitrogen, deprotonated amide nitrogen, and carboxylate oxygen donors (*III*). Within the investigated systems the species $[CuL_2H_2]$ and $[CuL_2]^{2-}$ could be excluded[8]. Also the ESR spectrum of the dimer complex $[Cu_2L_2(OH)]^{-}$ is not detectable in aqueous solution at room temperature. The typical ESR spectrum recorded at 103 K is shown in Fig. 3. The use of various dipeptides does not lead to any significant changes in the form of the spectrum. Changes in g and A values are rather small.

Based on the $g_{\parallel}$, $g_{\perp}$, g_0, and A_0 values (Table I) it appears that binary complexes fall in two well-distinct groups: the amino acid complexes are characterized by $g_{\parallel} > 2·26$, $g_{\perp} > 2·06$, $g_0 > 2·15$, and $A_0 \leqq 63 . 10^{-4} cm^{-1}$ whereas dipeptide complexes (with the exception of the GlyPro) exhibit lower g factors and higher A_0 values. Likewise, the electronic spectra show absorption maxima (Table II) at energy values which are higher for the dipeptide than for the amino acid complexes.

Inspecting Table I, one can see that the ESR parameters of the 1 : 1 Cu(II)-GlyPro system are similar to those for $[CuL']^+$ complexes. Its electronic spectrum shows absorption maximum at energy values which are even lower than those for parent amino acid complexes. Since proline contains the imino group, there is no ionizable amide-NH-proton when it is inserted into a peptide chain; hence the proline-nitrogen is unable to bind metal ions As a result the proline residue in any position other than the N-terminal position may act as a "break-point" to metal coordination[1], allowing the two ends of the peptide chains to behave independently. As a consequence of this fact large (rather unfavored eight-membered) ring can be formed.[14] Consequently, the GlyPro acts as a bidentate ligand.

TABLE II

Ligand field energies and molecular orbital coefficients for the studied complexes

Complex	ΔE_{xy}, cm^{-1}	α^2	α'^2	β_1^2	β^2	ε'^2
$[Cu(Gly)]^+$	14 684	0·779	0·312	0·930	0·873	0·259
$[Cu(Pro)]^+$	14 771	0·769	0·324	0·863	0·866	0·248
$[Cu(Phe)]^+$	14 641	0·703	0·400	0·820	0·851	0·178
$[Cu(Try)]^+$	15 528	0·712	0·385	0·855	0·839	0·188
$[Cu(His)]^+$	14 881	0·771	0·322	0·899	0·777	0·250
$[Cu(Hm)]^{2+}$	14 970	0·740	0·355	0·885	0·928	0·217
$[Cu(en)]^{2+}$	15 600	0·752	0·342	0·907	0·748	0·230[a]
[Cu(GlyGly)]	15 267	0·660	0·440	0·823	0·582	0·132
[Cu(GlyLeu)]	15 361	0·635	0·466	0·832	0·653	0·106
[Cu(GlyTry)]	15 576	0·645	0·456	0·841	0·579	0·117
$[Cu(GlyPro)]^+$	13 947	0·705	0·393	0·872	0·885	0·167

[a] Value calculated according to Eq. (7).

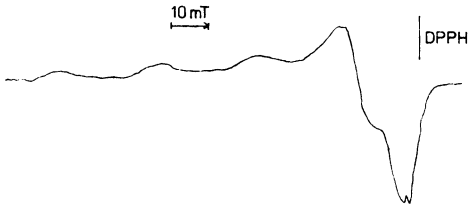

FIG. 3

The anisotropic ESR spectrum of the [Cu(GlyGly)] complex at 103 K

Bonding Parameters

The bonding parameters of the Cu(II) complexes are given in Table II. The values of α^2 remain for all the complexes within the narrow range of 0·63 – 0·78, indicating that σ-bond in the equatorial plane of the complex is of a covalent nature. However, since the overlap integrals in the B_{1g} orbital function are rather large, this bond cannot be unequivocally classified as covalent or ionic; it may only be assumed that the lower the value of α^2, the more covalent the nature of the bond. It can be thus concluded that the covalent nature of the copper–ligand in-plane σ-bond of the binary complexes is, within the investigated systems, decreasing in the sequence dipeptides > amino acids.

The overlap integrals in B_{2g} orbital function are small, therefore β_1^2 is a direct index of the covalency of the in-plane π-bonds[11]. The differences in β_1^2 values (0·82 – – 0·93) are not too much pronounced as those in α^2. The β^2 indicates the out-of-plane π-bonding range of 0·75 – 0·93.

The results are summarized in Fig. 4, where the ε'^2 values are plotted against α^2. The solid line represents the best linear fit to data points for complexes with effective D_{4h} symmetry (Eq. (7)).

$$\varepsilon'^2 = 1{\cdot}07\alpha^2 - 0{\cdot}575\,, \qquad (7)$$

where $r_{xy} = 0{\cdot}997$ and $S = 99{\cdot}4\%$.

This linear correlation can be attributed to a competition between different types of bonds (the first formed with the $3d_{x^2-y^2}$, the other with the 4s orbital) and has been found for several types of copper(II) complexes[13].

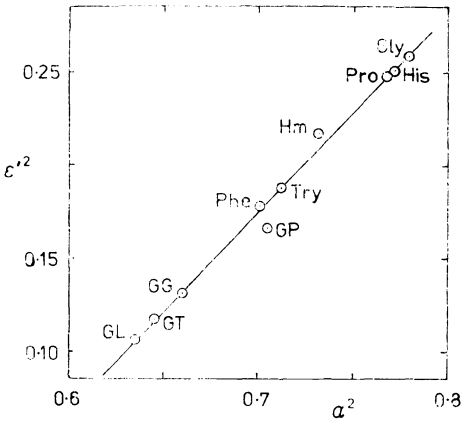

FIG. 4

Plot of ε'^2 vs α^2 for the binary copper(II) complexes of α-amino acids and dipeptides, respectively. GG, glycylglycine; GL, glycyl--L-leucine; GP, glycyl-L-proline; GT, glycyl--L-tryptophan

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