Multi-Microwave Frequency EPR in the Structural Characterization of Copper(II) Dipeptide Complexes

Rebecca Pogni, Giovanni Della Lunga, and Riccardo Basosi*

Contribution from the Department of Chemistry, University of Siena, Pian dei Mantellini 44, 53100 Siena, Italy. *Received April 6, 1992*

Abstract: Because of the multiplicity of possible equilibria in metal–peptide solutions, a new study using multifrequency EPR in combination with computer simulations and second-derivative displays was attempted. This approach permitted the determination with good precision of a set of spin Hamiltonian input parameters for the Cu–GlyHis and Cu–HisGly complexes. Two different arrangements in the first coordination sphere of copper were determined. In the case of the Cu–GlyHis complex, the simultaneous presence of the monomer and the bis complex and their relative percentages were established. For the Cu–HisGly complex, histamine-like coordination is proposed with a limited deviation from planarity. Spectral results obtained at room temperature for fast-tumbling copper complexes provided confirmation of the biological validity of the models. Molecular orbital coefficients characteristic of metal–ligand bonds were derived for effective D_{4h} symmetry. The influence of the coordination modes on the covalent and ionic character of the metal–ligand bonds is discussed.

Introduction

Dipeptides formed from amino acids containing side chains with potential donor centers are extremely important in biological systems. Among such dipeptides, those containing histidine, His, are particularly interesting since the imidazole group in histidine contains a labile proton which ionizes in the intermediate pH region.[1] The involvement of imidazole nitrogens is a crucial step in the coordination of histidine to copper to form stable chelate complexes in solution.[2]

The importance of the histidyl residues of proteins in the binding of copper(II) has been emphasized by Sundberg and Martin, who drew attention to the necessity of clarifying the mode of coordination of the simplest histidine-containing dipeptides.[3] Furthermore, many low-molecular-weight Cu^{2+} chelates using amino acids and peptides as ligands have been found to catalyze superoxide dismutation.[4-6]

The copper complexes of histidine and histidine-containing peptides, occurring naturally in blood plasma,[3,7] can be regarded as models for interactions between metal ions and proteins if the proper ligand-to-metal ion ratio is considered and physiological conditions are respected. Actually, the chief storage site of copper in the blood of mammals is ceruloplasmin, a protein containing eight copper atoms per molecule, but most non-ceruloplasmin copper in the blood is complexed to albumin or histidine, one of the strongest metal ion coordinating amino acids. Furthermore, a low-molecular-weight peptide-containing histidine or even histidine itself might compete with ceruloplasmin for copper in the blood, significantly altering free-ligand levels. For all the above reasons, the coordination behavior of histidine-containing dipeptides toward copper has seen a great deal of investigation.[1-3,7-10]

Coordination compounds of copper(II) typically consist of four nearby donor atoms arranged approximately in a plane about the metal ion, with the possibility of one or two, more distant, axial donors. It has been clearly stated that the binding of histidine-containing dipeptides and of histidine itself to copper is strongly dependent on pH, the ligand-to-metal ion ratio, and even the physical state of the sample. However, because of the multiplicity of possible equilibria in metal–peptide solutions, certain conclusions are still controversial.[1,2,8] After a large variety of experiments, we decided to focus our attention on the coordination behavior of the two complexes of copper with glycylhistidine (GlyHis) and histidylglycine (HisGly). Here, we consider only the physiological pH conditions for complexes with a 1:2 metal:peptide molar ratio in aqueous solution although all the pH ranges and a variety of different molar ratios have been explored.

EPR spectroscopy has been widely used to study complexes formed in solution between the copper(II) ion and various ligands, including α-amino acids.[11-14] Multifrequency EPR analysis used in correlation with computer simulations of spectra can also be a very useful tool in determining the chemical structure and molecular dynamics of copper in biological systems, mainly due to the existence of an optimal microwave frequency at which the superhyperfine structure is best resolved.[15-17] In the present study, the EPR spin Hamiltonian parameters (determined by a precise method) were used to determine the arrangement of the two complexes in the first coordination sphere and the covalent character of the metal–ligand bonds by multifrequency EPR spectroscopy used in combination with computer simulations.

Experimental Section

Glycyl-L-histidine and L-histidylglycine from Sigma Chemical Co. were used without further purification. Solutions were prepared with distilled water, and the uncorrected pH was adjusted with HCl or NaOH and determined with an "LCD" Model pH meter. Isotopically pure ^{63}CuO (from Oak Ridge National Laboratory, Oak Ridge, TN) was used for the EPR experiments. Stock solutions with a 1:2 molar ratio were prepared; [Cu] = 10^{-2} M and [peptide] = 2×10^{-2} M. The effective concentration of Cu^{2+} in the experiments was 10^{-3} M. A few drops of ethylene glycol were added to the solutions to obtain a better quality glass.

X-band EPR spectra were obtained with a Bruker 200D SRC X-band spectrometer, and S-band spectra were obtained with a microwave bridge from Medical Advances Inc., Milwaukee, WI. All the bridges were equipped with loop-gap resonators (Jagmar, Krakow, Poland) operating at ν = 9.5 GHz for the X-band bridge and ν = 4.04 GHz for the S-band bridge. Microwave frequencies were measured with an XL Microwave Model 3120 counter. The spectrometer was interfaced with a PS/2

* The author to whom correspondence should be addressed.

(1) Brookes, G.; Pettit, L. D. *J. Chem. Soc., Dalton Trans.* **1975**, 2112.
(2) Agarwal, R. P.; Perrin, D. D. *J. Chem. Soc., Dalton Trans.* **1975**, 268.
(3) Sundberg, R. J.; Martin, R. B. *Chem. Rev.* **1974**, *74*, 472.
(4) Joester, K. E.; Jung, G.; Weber, U.; Weser, U. *FEBS Lett.* **1972**, *25*, 25.
(5) Weser, U. *Struct. Bonding* **1973**, *17*, 1.
(6) Brigelius, R.; Spottl, R.; Bors, W.; Lengfelder, E.; Saran, M.; Weser, U. *FEBS Lett.* **1974**, *47*, 72.
(7) Sigel, H. *Met. Ions Biol. Syst.* **1973**, 63.
(8) McPhail, D. B.; Goodman, B. A. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 3683.
(9) Gubler, C. J.; Lahey, M. E.; Cartwright, G. E.; Wintrobe, M. M. *J. Clin. Invest.* **1953**, *32*, 405.
(10) Williams, D. R.; Furnival, C.; May, P. M. In *Inflammatory Diseases and Copper*; Sorenson, J. R. J., Ed.; Humana Press: Clifton, NJ, 1982; p 45.
(11) Szabó-Plánka, T.; Rockenbauer, A.; Gyor, M.; Gaizer, F. *J. Coord. Chem.* **1988**, *17*, 69.
(12) Basosi, R.; Valensin, G.; Gaggelli, E.; Froncisz, W.; Pasenkiewicz-Gierula, M.; Antholine, W. E.; Hyde, J. S. *Inorg. Chem.* **1986**, *25*, 3006.
(13) Pasenkiewicz-Gierula, M.; Froncisz, W.; Basosi, R.; Antholine, W. E.; Hyde, J. S. *Inorg. Chem.* **1987**, *26*, 801.
(14) Antholine, W. E.; Basosi, R.; Hyde, J. S.; Lyman, S.; Petering, D. H. *Inorg. Chem.* **1984**, *23*, 3543.
(15) Froncisz, W.; Hyde, J. S. *J. Chem. Phys.* **1980**, *73*, 3123.
(16) Hyde, J. S.; Froncisz, W. *Annu. Rev. Biophys. Bioeng.* **1982**, *11*, 391.
(17) Bednarek, J.; Schlick, S. *J. Am. Chem. Soc.* **1991**, *113*, 3303.

0002-7863/93/1515-1546$04.00/0

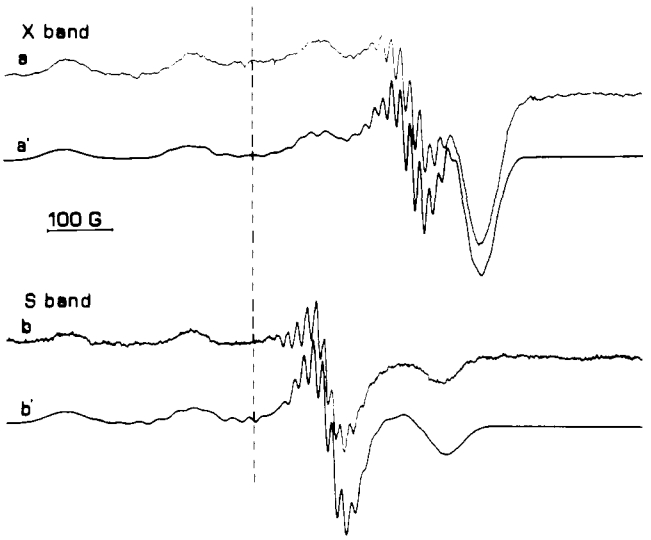

Figure 1. Experimental (a) X-band (ν = 9.5907) and (b) S-band (ν = 3.9856) EPR spectra of the ^{63}Cu(GlyHis)$_2$ complex in H_2O solution at pH = 7.3 at liquid nitrogen temperature (100 K) compared with (a', b') simulated EPR spectra of the same complex. Magnetic parameters are reported in Table I.

Technical Instruments Hardware computer, and the data were acquired and evaluated using the EPR data system CS-EPR produced by Stelar Inc., Mede, Italy.

The CUKOS program for the simulation of EPR spectra of fast-tumbling copper complexes in an isotropic environment written in Quick Basic is based on Kivelson's theory of line width[18] and includes the second-order shift equation of Bruno et al.[19] and the further assumption of Lorentzian line shapes. With the input parameters g_{iso}, Δg, A_{iso}, ΔA, and the rotational correlation time τ_c, the program calculates the paramaters of Kivelson's equations, the line widths, line positions, and line shapes. It is also able to accommodate two sets of nitrogens with up to four in each set. The program was devised to take into account the simultaneous presence of multiple species in solution. The residual width, including inhomogeneous contributions, allows a convolution between Lorentzian and Gaussian line shapes to be considered in the computer simulations.[20] A Monte Carlo calculation method was added to the program. The Monte Carlo method randomly varied selected spectral parameters within defined limits in order to fit experimental data. The parameters g_{iso}, A_{iso}, and A^N_{iso} were taken from room-temperature X-band and S-band experimental data.

The computer program CUSIMNE, a modified version written in MS-Quick Basic of a program by J. R. Pilbrow, Monash University, Clayton, Victoria, Australia, was used for the simulations of frozen-solution spectra of cupric complexes.[22] The program includes separate tensors for g values and the A^{Cu}, $A^N(1)$, $A^N(2)$, $A^H(1)$, and $A^H(2)$ hyperfine interactions, where the symbol $A^X(I)$ denotes the interaction with the Ith nucleus of type X.

The simulations for both programs were obtained using a Compaq Deskpro 486/50L with an 8-megabyte memory and a 50-MHz clock. The simulations were performed until further changes in the EPR parameters did not improve the quality of fit.

Results

1. EPR Spectra. X- and S-band EPR spectra for Cu–GlyHis are presented in Figure 1, and those for Cu–HisGly are presented in Figure 2. The broad lines in the parallel region are direct evidence of N-ligation, because of the unresolved shf structure. Furthermore, local structural inhomogeneities ("strain") can lead to a distribution in the g and hyperfine tensors causing line broadening.[15-17,21] In the perpendicular region, Figures 1 and 2 show a striking difference in hyperfine resolution at both X- and S-bands. Unfortunately, the perpendicular region is difficult to interpret without resolution of superhyperfine structure in the $g_{\parallel}$ region. The lack of resolved structure for Cu–HisGly in Figure 2 arises from the singular interplay of perpendicular features of magnetic parameters of Cu(II) and nitrogen ligands (a feature not shared by Cu–GlyHis). The superimposition of spectra from two species in the experimental spectra of Figure 1 may explain the imperfect fit with the simulation in Figure 1b'.

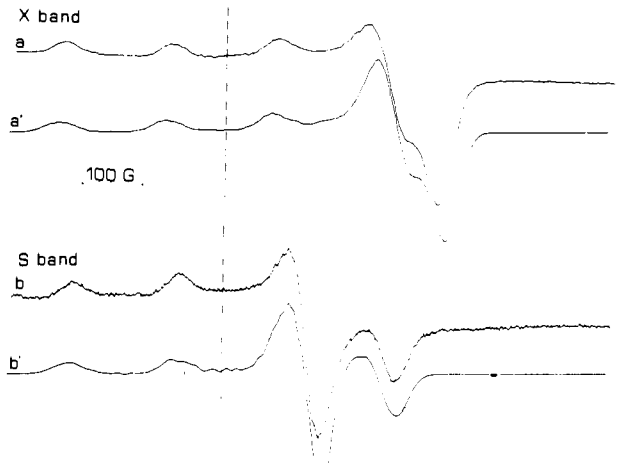

Figure 2. Experimental (a) X-band (ν = 9.5897) and (b) S-band (ν = 3.9895) EPR spectra of the ^{63}Cu(HisGly)$_2$ complex in H_2O solution at pH = 7.3 at liquid nitrogen temperature (100 K) compared with (a', b') simulated EPR spectra of the same complex. Magnetic parameters are reported in Table I.

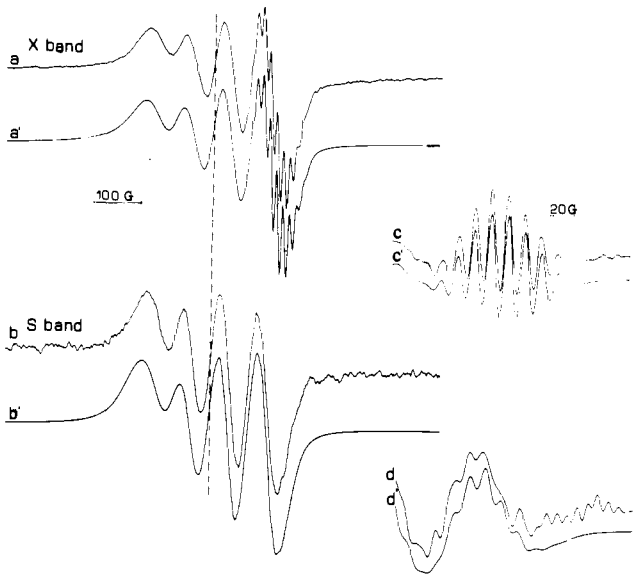

Figure 3. (a, b) Experimental and (a', b') simulated EPR spectra of the ^{63}Cu(GlyHis) complex in aqueous solution at pH = 7.3 at room temperature obtained at X-band (top) and S-band (bottom). (c, d) 200-G expansion of the second-derivative $M_I = +3/2$ copper component at X- and S-bands paired with their simulations (c', d'). Spectra were simulated considering the simultaneous presence of multiple species in solution (38% bis complex and 62% monomer). EPR parameters for the bis complex are reported in Table I. For the monomer, g_{iso} = 2.1134, A_{iso} = −0.0068 cm^{-1}, and A^N_{iso} = −0.0012 cm^{-1}.

The EPR spectra of fast-tumbling copper complexes, obtained at X- and S-bands, are shown in Figure 3 for Cu–GlyHis and in Figure 4 for Cu–HisGly paired with simulated spectra and the expanded second derivative of the fourth component $M_I = +3/2$. The EPR spectra of fast-tumbling copper complexes in an isotropic environment show superhyperfine structure arising from the coupling of ^{14}N in the first coordination sphere of copper. The different features of the two complexes are clearly revealed by the EPR spectra recorded in frozen and liquid solutions at

(18) Wilson, R.; Kivelson, D. *J. Chem. Phys.* **1966**, *44*, 4445, 154.
(19) Bruno, G. V.; Harrington, J. K.; Eastman, M. P. *J. Phys. Chem.* **1977**, *81*, 1111.
(20) Basosi, R.; Antholine, W. E.; Froncisz, W.; Hyde, J. S. *J. Chem. Phys.* **1984**, *81*, 4849.
(21) Hyde, J. S.; Pasenkiewicz-Gierula, M.; Basosi, R.; Froncisz, W.; Antholine, W. E. *J. Magn. Reson.* **1989**, *82*, 63.
(22) Rakhit, G.; Antholine, W. E.; Froncisz, W.; Hyde, J. S.; Pilbrow, J. R.; Sinclair, G. R.; Sarkar, B. *J. Inorg. Biochem.* **1985**, *25*, 217.

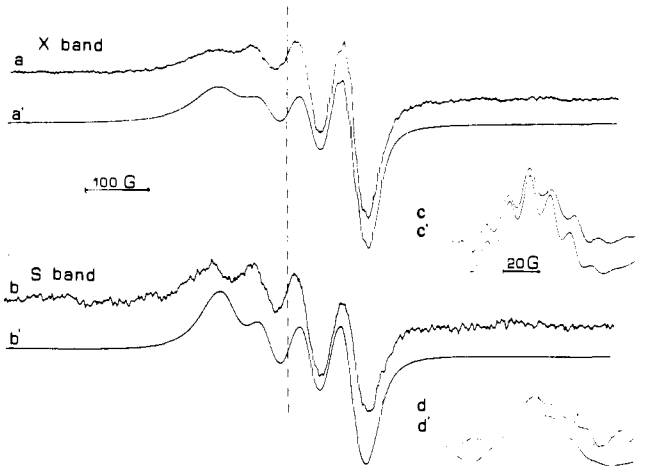

Figure 4. (a, b) Experimental X- and S-band EPR spectra of the ^{63}Cu-(HisGly)$_2$ complex in H_2O solution at pH = 7.3 at room temperature. (a', b') Simulated ESR spectra of the same complex obtained with the magnetic parameters reported in Table I. (c, d) 200-G expansion of the second-derivative $M_I = +^3/_2$ copper component at X- and S-bands paired with their simulations (c', d').

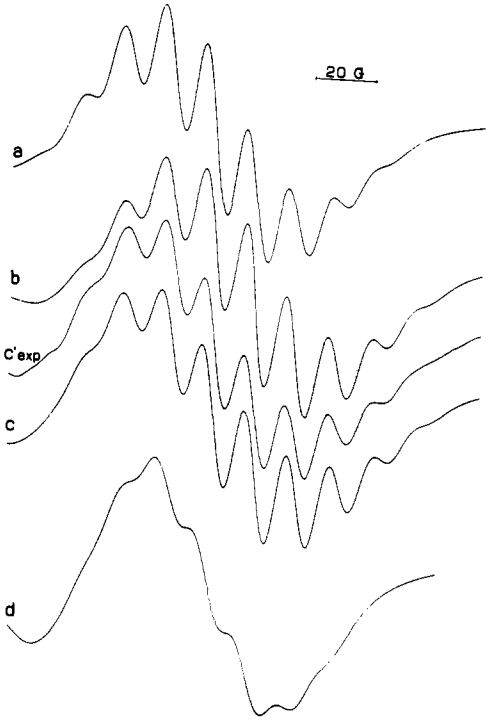

Figure 5. (c') Experimental 200-G expanded EPR spectrum of the $M_I = +^3/_2$ copper component for the ^{63}Cu–GlyHis complex in aqueous solution at pH = 7.3 at 298 K. (a) Simulated spectrum obtained for the case of four-^{14}N-ligation. (b) Simulated spectrum for 62% four ^{14}N and 38% three ^{14}N. (c) Simulated spectrum for 62% three ^{14}N and 38% four ^{14}N. (d) Simulated spectrum for the case of three ^{14}N.

physiological pH and with a 1:2 Cu:ligand molar ratio.

Figure 5 shows the expansion of the $M_I = +^3/_2$ component for the complex Cu–GlyHis in a fast-tumbling regime as in Figure 3. The experimental spectrum (Figure 5c') is paired with the simulations for a different number of ligand nitrogens. The procedure proposed[31] is based on the relative intensities of the patterns for four (Figure 5a) vs three nitrogens (Figure 5d). In this procedure, the simulations are adjusted until the best fit of the central three lines is obtained using three or four nitrogens. The best simulation obtained was that in which we considered the simultaneous presence of a species with four nitrogens and another with three nitrogens as shown in Figure 5c. Figure 5b shows the arrangement where the four-nitrogen species is predominant. All the patterns have been simulated with the proper τ_c for monomeric and bis-complex species.

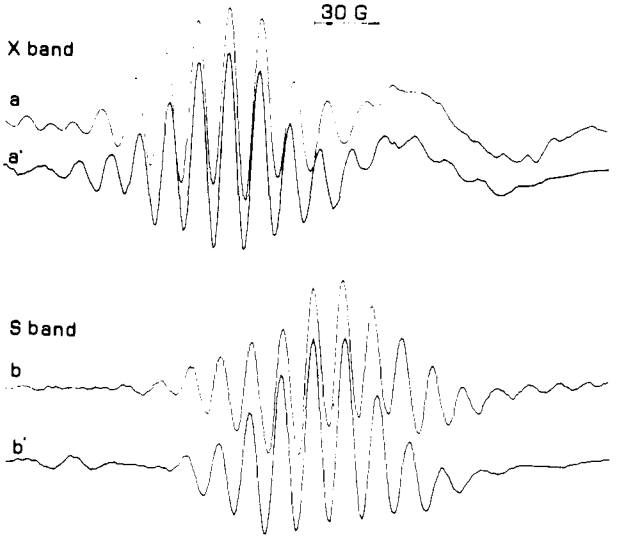

Figure 6. (a, b) 300-G expansion of $g_\perp$ region second-derivative EPR spectra at the X- and S-bands of Figure 1 paired with (a', b') simulated EPR spectra obtained with the magnetic parameters reported in Table I.

2. Simulations. Experimental multifrequency EPR analysis combined with computer simulations of spectra at frozen-solution and room temperature is a useful tool for determining the chemical structure of copper biological systems, due to the existence of an optimal microwave frequency at which the superhyperfine structure is best resolved. In general, the requirement of a good fit at different frequencies for EPR spectra of copper imposes some constraints on the precision of the EPR parameters, and it is a critical test of the theory. In calculating the spectra, we assumed axial **g**, hyperfine, and superhyperfine tensors, with a common principal axis system. In the first approximation, all ^{14}N ligands were considered magnetically equivalent. Programs for simulation were able to consider a convolution of Gaussian and Lorentzian line shapes, chosen to obtain the best fit.

The process of simulation was initiated by reading the approximate values of all parameters from the spectra. The **g**-tensor values were adjusted by fitting the spectra at X-band with the highest sensitivity to these changes. The simulations for liquid-phase EPR spectra started with a Monte Carlo calculation method. This method randomly varied selected spectral parameters within defined limits in order to fit experimental data. When a good fit was obtained at one frequency, the magnetic parameters were tested at the other frequency, and the parameters were varied by an iterative process until the best fit was obtained at both X- and S-bands for the two physical states of the samples. The use of second-derivative displays was a crucial step in obtaining a good set of parameters. In Figure 6a, the second-derivative (or second-harmonic) display emphasizes sharp features and discriminates against broad features. This display is particularly useful for analyzing superhyperfine patterns. Shoulders in the spectrum become peaks with precisely defined turning points that are useful for accurate measurements of coupling constants. The analysis of the perpendicular region is particularly complicated because of spectral overlap. As usual, the perpendicular component is a "hodgepodge" of the perpendicular components of copper, the forbidden transitions depending on the copper quadrupole interaction, which varies greatly depending on ligation and the ^{14}N hyperfine couplings, and finally the $\perp$ region is dominated under a wide range of conditions by so-called extra absorption or "overshoot" lines arising from an interplay of g and A anisotropies. For all of these reasons, the perpendicular region of the spectrum is always difficult to simulate. Figure 6 shows the expanded second-derivative display of the $\perp$ region obtained at X- and S-bands using the same input set of magnetic parameters. In many systems, the Peisach–Blumberg diagrams[23] can be used to deduce

(23) Peisach, J.; Blumberg, W. E. *Arch. Biochem. Biophys.* **1974**, *165*, 691.

the ligation scheme because the number of ^{14}N ligands is related to a decrease in the $g_{\parallel}$ value and an increase in the $A_{\parallel}$ value with respect to Cu^{2+} bound to oxygen only. For four-nitrogen ligands, these diagrams predict $g_{\parallel} < 2.3$ and $A_{\parallel} > 0.0160$ cm^{-1}. This is in good agreement with our results for the coordination proposed here for both complexes.

The spin Hamiltonian parameters that eventually were used to get the best fit are given in Table I. The signs of the perpendicular components of the copper hyperfine interaction were chosen so that isotropic A parameters obtained from the room-temperature and 110 K spectra were in agreement.

3. Discussion. The two experimental EPR spectra in frozen solution paired with their simulations (Figures 1 and 2) can be described by an axial spin Hamiltonian

$$\mathcal{H} = \beta[g_{//}H_zS_z + g_{\perp}(H_xS_x + H_yS_y)] + [A_{//}S_zI_z + A_{\perp}(S_xI_x + S_yI_y)] + \sum_m \overline{SA^N}\overline{I}_m \quad (1)$$

where the symbols have their usual meanings. The g values and hyperfine splittings depend on the **g** and **A** matrices respectively; $\sum_m \overline{SA^N}\overline{I}_m$ represents the interaction between the unpaired electron and the Nth ligand nucleus.

The magnetic parameters obtained for the two complexes in the $g_{\parallel}$ region are in agreement with values of $g_{zz} > g_{xx,yy} > 2.040$, suggesting that the complexes have a $d_{x^2-y^2}$ ground state characteristic of square planar, square-base pyramidal, or octahedral stereochemistry.[24,25] Close comparison of $g_{\parallel}$ and $A_{\parallel}$ values of the Cu–GlyHis complex and the homologue values of the Cu–HisGly complex in Table I reveals an increase in $g_{\parallel}$ from 2.223 to 2.247 and a simultaneous decrease of $A_{\parallel}$ from −0.0200 to −0.0186 cm^{-1}. This trend can be explained by limited distortion from square planar symmetry of the equatorial plane for Cu–HisGly. Furthermore, differences in the overall charges of the two complexes may account for differences in the EPR spectra.[23]

Multifrequency EPR spectra recorded at room temperature are crucial for the biological significance of the model (Figures 3 and 4). When combined with computer simulations, they minimize the problems arising from the relative lack of details of the experimental features and permit a more sensitive determination of the rotational correlation time (τ_c), the reorientational time of the complex modulating the energies related to g and A anisotropies. The values obtained for the Cu–GlyHis and Cu–HisGly complexes of Figures 3 and 4 are reported in Table I. The difference between the two values ($\tau_c = 51.8 \pm 10$ ps for Cu–GlyHis and $\tau_c = 44.0 \pm 10$ ps for Cu–HisGly) is within the limits of experimental error and can hardly be regarded as a significant difference between the two complexes. In general, the correlation times were usually found to be much shorter than those calculated by the Debye–Stokes formula.[26] The comparison of experimental room-temperature Cu–GlyHis spectra at X- and S-bands reported in Figure 3 shows a clear difference in the resolutions of the superhyperfine structures on the $M_I = +^3/_2$ copper component at two frequencies. The X-band spectrum is much better resolved than that of the S-band. The fourth component of the expanded second derivative was very useful for determining an accurate measure of the isotropic ^{14}N coupling constant (Figure 3a′,b′). The relative lack of intensity of the ^{14}N superhyperfine pattern of the complex Cu–HisGly in Figure 4a is indicative of a smaller value of the ^{14}N coupling constant, appreciable in the second-derivative display (Figure 4a′,b′).

The ^{14}N hf coupling constants may be useful for estimation or confirmation of the structure of copper-binding sites. A correlation has been demonstrated between the superhyperfine coupling constants of the donor nitrogens in the $g_{\parallel}$ parts of the spectra and the structure of the coordination sphere in an ^{14}N ENDOR study

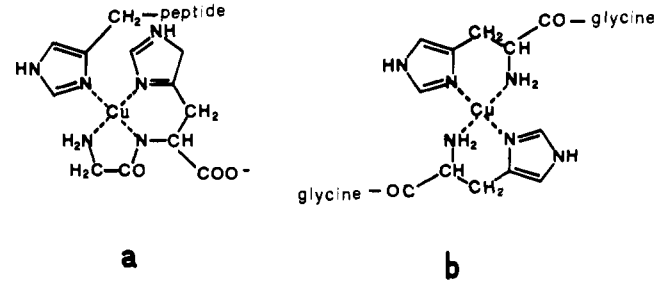

Figure 7. Tentative structures proposed for (a) $^{63}Cu(GlyHis)_2$ and (b) $^{63}Cu(HisGly)_2$ complexes in aqueous solution at pH = 7.3.

of a large number of copper complexes.[27] The hf coupling constants of nitrogens in the first coordination sphere correlate well with the donor sets in the equatorial plane as well as with other kinds of nitrogens, such as deprotonated amide nitrogens, aromatic aza nitrogens, or amine nitrogens. Furthermore, the ^{14}N hf coupling constants are affected by hybridized states of the nitrogen orbitals; i.e., nitrogens with planar conformations such as deprotonated amide nitrogens and aromatic aza nitrogens have larger coupling constants than nitrogens with tetrahedral conformations such as amine nitrogens.[27] Taking the coordinating orbitals of deprotonated amide and aromatic aza nitrogens to be pure sp^2-hybridized orbitals and those of amine nitrogens to be pure sp^3, the spin densities on nitrogens in the sp^3 state are slightly less than those on the nitrogens in the sp^2 state. This is in good agreement with the observed trend that nitrogens having planar conformations, sp^2 type conformations, show larger hf coupling constants than nitrogens having tetrahedral, sp^3, conformations. Distortion from the planar array to the tetrahedral array in the first coordination sphere results in a reduction in the overlap of the copper unpaired electron orbital with the ligands, causing a decrease in the nitrogen coupling constants. In the case of the Cu–HisGly complex, if excess HisGly is present, bis-complex formation in which deprotonation of the peptide–NH linkage is suppressed is probable.[28] Voelter et al. confirmed this hypothesis with ^{13}C NMR experiments at pH 7, at which the glycine moiety is not involved in copper ligation.[29] The ligation suggested was pure histamine-like coordination with two imidazole nitrogens and two amine nitrogens in the first coordination sphere of copper. This arrangement is in agreement with our EPR results and with the EPR results previously obtained for histidine.[12,13] In fact, in the case of Cu–HisGly, the carboxyl group is blocked by peptide bonding in favor of a total histamine-like coordination, while, in the histidine complex, a mixture of histamine-like and glycine-like structures has been proposed on the basis of experimental findings.[12,13,29]

In the case of the Cu–GlyHis complex, the geometrical positions of the donor atoms in GlyHis make the coordination of three nitrogens (amino, peptide, and imidazole) possible, which results in a very stable monomeric complex.[28] According to previous crystallographic studies,[30] these three nitrogen atoms are all approximately the same distance (2.0 Å) from the copper ion while charge neutralization is achieved by a carboxyl oxygen from another molecule of ligand. The presence of an excess of ligand determines the appearance of the bis complex because the free coordination site in the monomer can be occupied by the N^3(imidazole) nitrogen of another GlyHis molecule. The bis-complex formation has been favored by increasing the concentration of GlyHis, thus avoiding the complexity of analyzing a mixture. We identified the monomeric species at lower pH (pH = 6 data not

(24) Hathaway, B. J.; Billing, D. E. *Coord. Chem. Rev.* **1970**, *5*, 143.
(25) Hathaway, B. J.; Tomlinson, A. A. G. *Coord. Chem. Rev.* **1970**, *5*, 1.
(26) Basosi, R. *J. Phys. Chem.* **1988**, *92*, 992.
(27) Iwaizumi, M.; Kudo, T.; Kita, S. *Inorg. Chem.* **1986**, *25*, 1546.
(28) Sóvágó, I.; Farkas, E.; Gergely, A. *J. Chem. Soc., Dalton Trans.* **1982**, 2159.
(29) Voelter, W.; Sokolowski, G.; Weber, U.; Weser, U. *Eur. J. Biochem.* **1975**, *58*, 159.
(30) Blount, J. F.; Fraser, K. A.; Freeman, H. C.; Szymanski, J. T.; Wang, C. H. *Acta Crystallogr.* **1967**, *22*, 396.
(31) Hyde, J. S.; Antholine, W. E.; Froncisz, W.; Basosi, R. In *Advanced Magnetic Resonance Techniques in Systems of High Molecular Complexity*; Niccolai, N., Valensin, G., Eds.; Birkhausen: Boston, MA, 1986; p 363.

Table I. Parameters Used to Simulate X-Band and S-Band EPR Spectra for Cupric Ion Complexes with Dipeptides[a]

system	g_{iso}	$g_{//}$	$g_{\perp}$	Δg	$A_{iso}(^{63}Cu)$	$A_{//}(^{63}Cu)$	$A_{\perp}(^{63}Cu)$	ΔA	$A^N_{//}$	$A^N{}_{\perp}$	A^N_{iso}	τ_c (ps)
^{63}Cu-GlyHis												
298 K	2.112			0.180	-0.0073			0.0211			-0.0013	51.8[b]
100 K		2.223	2.053			-0.0200	-0.0011		-0.0015	-0.0013		
^{63}Cu-HisGly												
298 K	2.114			0.235	-0.0053			0.0194			-0.0010	44.0[b]
100 K		2.247	2.044			-0.0186	+0.0008		-0.0013	-0.0010		

[a] Values for the hyperfine and superhyperfine splittings are given in cm^{-1}. [b] Correlation times in ps (10^{-12} s). ^{63}Cu magnetogyric ratio 0.709 04 s^{-1} $G^{-1} \times 10^{-4}$.

shown) and obtained the best fit of Figure 3, considering the concomitant presence of the bis complex and the monomeric species in a ratio of 38% to 62%.

On the basis of above discussion, the structures reported in Figure 7 are proposed.

4. Calculation of Bonding Parameters from EPR Data. Assuming the EPR parameters from Table I, using the theoretical basis of Maki and McGarvey[32] for copper(II) complexes, it is possible to apply a semiempirical LCAO–MO scheme and express anisotropic g values and hyperfine constants as a function of molecular-orbital coefficients and certain atomic constants.

The construction of one-electron molecular orbitals reflects the local symmetry of copper(II) complexes, and we can hope to obtain interesting information about the differences previously detected for the homologous dipeptides Cu–GlyHis and Cu–HisGly. Usually, for copper, the general symmetry corresponds to an octahedral structure with strong tetragonal elongation, but additional rhombic or tetrahedral distortion can arise from differences in donor atoms of the ligand or steric repulsion between chelating molecules. Chemical considerations and previous studies[33] suggest that it is reasonable to confine ourselves to the case of a tetragonally distorted octahedral effective D_{4h} symmetry. In this case, the antibonding molecular orbitals of the copper(II) ions can be written[11,32] as shown in (2)–(6).

$$\psi_{b_{1g}} = \alpha\, d_{x^2-y^2} - \alpha\varphi_L(x^2 - y^2) \quad (2)$$

$$\psi_{a_{1g}} = \alpha_1 d_{z^2} - \alpha_1'\varphi_L(z^2) \quad (3)$$

$$\psi_{b_{2g}} = \beta_1 d_{xy} - \beta_1'\varphi_L(xy) \quad (4)$$

$$\psi_{e_g} = \beta d_{xz} - \beta'\varphi_L(xz) \quad (5)$$

$$\psi_{e_g} = \beta d_{yz} - \beta'\varphi_L(yz) \quad (6)$$

The x and y axes are directed toward the donor atoms of the ligands. The d and φ_L functions represent the copper(II) 3d orbitals and the ligand group orbitals of appropriate symmetry, respectively. In the case of octahedral structures, the unpaired electron is placed in the $\Psi_{b_{1g}}$ antibonding orbital. These equations can be related to the spin Hamiltonian parameters as follows:

$$A_{//} = P\left(-k - \frac{4\alpha^2}{7} + (g_{//} - 2.0023) + \frac{3(g_{\perp} - 2.0023)}{7}\right) \quad (7)$$

$$A_{\perp} = P\left(-k + \frac{2\alpha^2}{7} + \frac{11(g_{\perp} - 2.0023)}{14}\right) \quad (8)$$

where P is taken as 0.036 cm^{-1} and hyperfine splittings are expressed in consistent units. These analytical equations can be used to measure the extent of ionic bonding (α^2) and the extent of the contact term (K) from the magnetic parameters in Table I.

The K value obtained for Cu–GlyHis is 0.31 compared to a value of 0.26 obtained for the complex Cu–HisGly. The K value is lower for Cu–HisGly than for Cu–GlyHis. The term K arises from the Fermi contact interaction which has its origin in a nonvanishing probability of finding the unpaired electron at the site of the nucleus. This term is assumed to be independent of the direction of the magnetic field, and the maximum value is attained at an intermediate covalency of α^2.[34] The K value for the Cu–HisGly complex is consistent with a tetrahedral distortion of the symmetry around the metal ion. This type of distortion can lead to 3d–4p hybridization,[35] which explains the reduction of the anisotropic hyperfine term.

The terms containing the factor α^2 arise from the dipole–dipole interaction between magnetic moments associated with the spin motion of the electron and the nucleus. This contribution is predicted to be reduced by delocalization of the unpaired electron on the neighboring atoms. Its value decreases with increasing covalency to a minimum theoretical value of 0.5, increasing to a maximum of 1.0 for a completely ionic copper–ligand bond. The values obtained for our complexes are $\alpha^2 = 0.84$ for Cu–GlyHis and $\alpha^2 = 0.90$ for Cu–HisGly. These values show a trend toward a more pronounced ionic character for the latter complex.

Conclusions

(i) Multifrequency EPR in combination with computer simulations and second-derivative displays permitted the determination of a set of spin Hamiltonian input parameters for the Cu–GlyHis and Cu–HisGly complexes.

(ii) Different arrangements in the first coordination sphere of copper were determined. In the case of the Cu–GlyHis complex, the simultaneous presence of the monomer and the bis complex and their relative percentages were well established. For the Cu–HisGly complex, histamine-like coordination was proposed.

(iii) Spectra of fast-tumbling copper complexes provided a stringent requirement for internal coherence of A values obtained at low temperature. The low value obtained for $A_{\perp}$ in the Cu–HisGly complex is consistent with a small displacement of the copper from the square planar configuration.

(iiii) Bonding parameters calculated from EPR data were used to determine the covalent and ionic characters in both cases. Data obtained suggest a square planar configuration with tetragonal distortion for the Cu–GlyHis complex and a limited but significant deviation from this symmetry for the Cu–HisGly complex.

Acknowledgment. This research was supported by a MURST fund (40%) and by the Italian National Council of Research (CNR) (Grants 9000285.03 and 9004084.12).

(32) Maki, A. H.; McGarvey, B. R. *J. Chem. Phys.* **1958**, *29*, 31, 35.
(33) Abragam, A.; Bleaney, B. In *Electron Paramagnetic Resonance of Transition Ions*; Clarendon Press: Oxford, U.K., 1970.
(34) Rockenbauer, A. *J. Magn. Reson.* **1979**, *35*, 429.
(35) Sharnoff, M. *J. Chem. Phys.* **1964**, *41*, 2203; **1965**, *42*, 3383.