Electron Paramagnetic Resonance Study of Weak Exchange Interactions between Metal Ions in a Model System: Cu^{II}Gly-Trp[†]

Antonio J. Costa-Filho,*,[‡] Otaciro R. Nascimento,[‡] and Rafael Calvo[§]

Departamento de Física e Informática, Instituto de Física de São Carlos, Universidade de São Paulo, C.P. 369, CEP 13560-970, São Carlos, SP, Brazil, and Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral and INTEC (CONICET-UNL), Güemes 3450, 3000 Santa Fe, Argentina

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The structure of the copper compound with the peptide Gly-Trp belongs to the space group $P_{21}2_{12}$, with Z = 4. The four chemically identical but magnetically nonequivalent Cu(II) molecules in the unit cell are weakly connected by a network of biologically relevant pathways involving hydrogen bonds and cation- π interactions. The magnitudes of the exchange interactions between copper ions (*J*) transmitted along these paths were evaluated from the angular variation of the line width and the collapse of the EPR lines observed at 9.5 and 35 GHz with the magnetic field in the three crystalline planes of oriented single-crystal samples. Values $|J|/k_{\rm B} = 3 \pm 1$ and 12 ± 3 mK were obtained for the couplings transmitted through bridges containing H-bonds, and $|J|/k_{\rm B} = 5 \pm 2$ mK for the interaction transmitted through bridges containing a cation- π contact. These magnitudes are analyzed in terms of the structural information about the intervening long chemical paths.

1. Introduction

Noncovalent intra- and intermolecular interactions play important roles in protein structure, receptor-ligand contacts, and enzyme-substrate binding.^{1,2} Hydrogen bonds,³ aromatic ring stacking,^{4,5} cation- π interactions,⁶ and other weak bonds stabilize the structure of the metal binding site or provide intermolecular contacts⁷ and electron-transfer paths.⁸ These bonds can be readily reproduced in low molecular weight molecules (model systems), provided by complexes of transition metal ions with amino acids or small peptides. Therefore, these model systems are convenient to study interactions taking place in biologically relevant systems.

Electron paramagnetic resonance (EPR) has been used for many years to study the electronic structure and the local properties of metal ions9,10 and radicals,11 and extensively applied to the study of proteins and other biological molecules.^{11,12} More recently, the properties of weak chemical paths connecting specific chromophores with unpaired spins have also been studied with EPR in model compounds13-15 and in proteins,^{16,17} evaluating the exchange interactions between the connected metal ions or radicals. These measurements have two purposes. On the one side the magnitude of the exchange interaction is related to the electronic structure of the path¹⁸ thus providing information about the bridge that is difficult to obtain when one is dealing with long noncovalent bonds, whose weak contributions are masked by stronger interactions. On the other hand, it has been shown that the magnitudes of the exchange interactions between unpaired spins are related to the matrix elements for electron transfer between redox centers in proteins,¹⁶ and thus measurements of the exchange couplings

[‡] Universidade de São Paulo.

give information about electron-transfer properties of the path that are difficult to obtain from other sources.⁸

We have shown in previous work that EPR experiments at room temperature in single crystal samples provide a powerful method to selectively evaluate the magnitudes of weak exchange interactions.¹⁹ Hence, in combination with crystal structure data, EPR studies of model compounds allow us to study the magnetostructural correlations needed to characterize properties of weak bonds. In principle, this information can be translated to similar chemical paths occurring in macromolecules. The method used to evaluate the exchange interactions stands on classical theories of exchange narrowing and collapse of resonances.^{20,21} The procedure to extract the information has been described^{15,19} and relies on careful EPR measurements of the angular variation of resonance positions and widths that are normally performed in single-crystal samples at room temperature.

Several types of exchange pathways such as carboxylate bridges,²² stacking of aromatic rings¹³ and hydrogen bonds,^{14,23} have been already studied by EPR. In the last years the importance of the cation– π interactions has been demonstrated for amino acids bearing aromatic side chains (Trp, Phe, and Tyr).⁶ There, the electronic cloud of a cation overlaps with the π orbitals of the aromatic ring, providing an interaction that can be as effective as the hydrogen bonds. The role of the cation– π interactions in transmitting exchange couplings between metal centers has been investigated recently by us in Cu^{II}Trp-Gly.¹⁵ In this model compound, cation– π contacts are responsible for exchange interactions with magnitudes $|J|/k_B \sim 60$ mK (k_B is the Boltzmann constant), which were evaluated by EPR in the presence of much larger exchange interactions transmitted through covalent carboxylate bridges.

An interesting model system, where H-bonds and cation $-\pi$ interactions connect Cu ions, is the compound aqua(glycyl-L-tryptophanato)copper(II) dihydrate [Cu^{II}Gly-Trp]. The structure of this compound was reported by Hursthouse et al.²⁴ The electronic structure of the copper ions was characterized by light

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^{*} Corresponding author. Phone: +55-16-273 9839. Fax: +55-16-271 5381. E-mail: ajcosta@if.sc.usp.br.

[§] Universidad Nacional del Litoral and INTEC.

absorption and EPR studies of aqueous solutions at different pH values and in single-crystal samples of Cu^{II}Gly-Trp.²⁵ No information was obtained in that work about the magnitudes of the interactions between Cu(II) ions, which is then the main purpose of this paper. We report here detailed room-temperature EPR measurements on a single-crystal sample performed at 9.5 and 35 GHz. As in the case of Cu^{II}Trp-Gly, in Cu^{II}Gly-Trp there are four molecules per unit cell, related by the symmetry operations of the space group P2₁2₁2₁. Unlike Cu^{II}Trp-Gly, Cu^{II}-Gly-Trp is of molecular type and does not show a spin chain structure produced by much stronger exchange interactions. In Cu^{II}Gly-Trp the paths containing cation $-\pi$ contacts compete only with paths containing hydrogen bonds. The exchange interactions transmitted through paths containing H-bonds and through cation $-\pi$ contacts in Cu^{II}Gly-Trp are evaluated in this work and the results are discussed in terms of the structure of these bonds.

2. Experimental Section

2.1. Sample Preparation. The compound Cu^{II}Gly-Trp [aqua-(glycyl-L-tryptophanato)copper(II) dihydrate, C₁₃H₁₅CuN₃O₄· 2H₂O] was obtained from the 1:1 stoichiometric ligand:metal reaction of aqueous solutions of the dipeptide glycyl-L-tryptophan (Sigma Chemical Co.) in NaOH and CuCl₂ at pH = 5.0, and at room temperature.²⁴ Elongated dark blue crystals with larger dimensions along the *a* crystal axis were obtained after several days of slow evaporation. They show an irregular morphology but have a well-defined *ab* plane that was used to orient the single crystal samples. A single crystal of approximately $2 \times 1 \times 1$ mm was glued to a cubic sample holder made of a cleaved KCl crystal, which defines the laboratory orthogonal axes ($xyz \equiv abc$) for the EPR experiments. The orientation of the sample in the sample holder was confirmed by X-ray diffraction techniques.

2.2. EPR Measurements. The EPR data were obtained at 9.5 and 35 GHz with a Varian E-109 spectrometer. The sample holder described above was mounted on top of a horizontal pedestal in the center of the microwave cavity. The angular variation of the EPR spectra with the magnetic field $\mathbf{B} = B\mathbf{h}$, with $\mathbf{h} = (\sin \theta \cos \varphi, \sin \theta \sin \varphi, \cos \theta)$, was measured in the ab = xy, ca = zx, and cb = zy planes of the sample, by either rotating the sample (at 9.5 GHz) or rotating the electromagnet (at 35 GHz). Only one resonance was observed in the ca and cb crystal planes, whereas two resonances were observed in the ab plane. From the observed spectra the angular variation of the squared g-factor and the line widths were evaluated in the three orthogonal planes ab, ca, and cb. The values of the squared g-factor measured at 9.5 and 35 GHz are displayed in Figure 1a,b, respectively. Figure 2a,b display the widths observed in the planes ca and cb, respectively, at 9.5 and 35 GHz. Figure 3 displays the widths observed in the ab plane at 35 GHz. The striking feature of the data displayed in Figure 2a,b is the large change of the line width with microwave frequency in the ca and cb planes. The results in Figures 1-3 were used to calculate the exchange couplings between copper ions.

3. Crystal Structure and Exchange Network of $Cu^{II}Gly$ -Trp

The crystallographic structure of Cu^{II}Gly-Trp was determined and refined by Hursthouse et al.²⁴ It crystallizes in the orthorhombic space group $P2_12_12_1$, with lattice parameters a = 7.74(4) Å, b = 13.78(3) Å, and c = 14.81(3) Å, and four molecules per unit cell (A, B, C and D, with A = (x, y, z), B = $(\frac{1}{2} - x, -y, \frac{1}{2} + z)$, C = $(-x, \frac{1}{2} + y, \frac{1}{2} - z)$, and D =



Figure 1. Angular variation of $g^2(\theta,\varphi)$ measured at (a) 9.5 GHz and at (b) 35 GHz in the three crystal planes of Cu^{II}Gly-Trp. The solid lines were obtained using the components of the g^2 tensor given in Table 1. The bottom axis represents the variation of the angle θ in the *ca* and *cb* planes and the angle φ in the *ab* plane. The positions of the *a*, *b* and *c* crystal axes of the sample are indicated by arrows.



Figure 2. Angular variation of the peak-to-peak line width ΔB_{pp} measured at 9.5 and 35 GHz (filled symbols) in (a) the *ca* crystal plane and (b) the *cb* plane of Cu^{II}Gly-Trp. The solid lines were obtained with the model described in the text. (c) includes the differences between the widths observed at 35 and 9.5 GHz.

 $(\frac{1}{2} + x, \frac{1}{2} - y, -z)$). These four molecules are chemically equivalent, but magnetically nonequivalent in the presence of



Figure 3. Angular variation of the peak-to-peak line width $\Delta B_{\rm pp}$ measured at 35 GHz in the *ab* crystallographic plane of Cu^{II}Gly-Trp. Information about the exchange couplings was obtained from the line width observed in the collapsed region, near the axes *a* and *b*.

an applied magnetic field. Sites B, C, and D are obtained from site A by 180° rotations around the *c*, *b*, and *a* axes, respectively, plus a translation. The copper ion has an approximately square planar coordination with the nitrogen atoms N1 and N2 from the amino group and peptide bond, respectively, and the oxygen atoms O2 and O4 from the carboxylate group and a water molecule, respectively, as equatorial ligands (see Figure 4 showing the labeling of the atoms). The complex is monomeric and the molecule behaves as a terdentate chelate via the copper ligands.

Hydrogen atoms were not located in ref 24, but their approximate positions can be inferred by considering the interatomic distances and angles. There is extensive hydrogen bonding between molecules due to the presence of coordinated water molecules that act as a hydrogen donor to the other two water molecules, each of which binds also to the carbonyl oxygen atoms. Hydrogen bonds also exist between the tryptophan nitrogen atom N3, and the carbonyl oxygen O3 of the carboxylic group of a neighbor molecule.

In the 3D spatial arrangement, each copper ion has indole rings from tryptophan residues at its two apical positions; one is from the same molecule and the other is from a neighboring molecule (Figure 5a). The angle between the normal to the plane of the square of ligands to copper and the normals to the planes of the indole rings from the same and the neighboring molecule are 50° and 13°, respectively. In the latter case, the planes are quite close, with the shortest distance being d(Cu-C4) = 3.12Å. The sum of van der Waals radii of Cu and C (estimated to be between 3.50 and 4.10 Å) is significantly larger than that distance, indicating an interaction between the copper ion and the aromatic ring.²⁴

Because the shortest distance between two copper ions is 7.74 Å, the overlap of copper atomic orbitals is negligible, and direct exchange interactions do not occur. Thus, the exchange interactions are transmitted through the diamagnetic paths bridging copper pairs (superexchange). To identify the chemical paths connecting nearest neighbor copper ions associated with each molecule in the unit cell, we analyze the interactions between a copper type A and the nearest coppers A, B, C, and D. All other nearest neighbor paths can be related to these by symmetry operations. A copper atom type A is connected to two nearest copper atoms type A at 7.74 Å along the *a* axis by two paths consisting of the hydrogen bonds N1^A–H–O3^A and O4^A–H–



Figure 4. (a) Molecular structure of $Cu^{II}Gly$ -Trp as reported in ref 24. The labeling of the atoms follows the Cambridge Structural Database and is different from that used in that work. One water oxygen (O6) is not included. (b) Overall packing of the four symmetry-related $Cu^{II}Gly$ -Trp in the unit cell (*cb* plane). Molecules are named A, B, C, and D according to the $P2_12_12_1$ symmetry relations. Dashed lines indicate some distances mentioned in the text.

 $O5^{A}$ -H-O3^A (in our notation N1^A is a nitrogen N1 atom belonging to a molecule type A).

A copper type A is connected to four neighbor type B, two at d = 8.377 Å, and two at d = 8.477 Å. These paths (Figure 6a,b) contain seven atoms including a sequence of two hydrogen bonds involving the oxygens O1, O4, O5, and O6 ($d(O4^{A}-O5W) = 2.697$ Å, $d(O4^{A}-O6W) = 2.654$ Å, $d(O1^{B}-O5W) =$ 2.723 Å, and $d(O1^{B}-O6W) = 2.900$ Å). The paths are very similar and have total bond distances d' = 11.90 Å and d' =11.77 Å.

Each copper type A is connected to two neighboring coppers type C by cation- π interactions between the copper ion and the indole ring of the tryptophan residue of the neighbor molecule, whose shortest distance is $d(Cu^A-C4C) = 3.120$ Å (see Figure 5a,b). This path includes six atoms and have a total bond length d' = 12.354 Å.

An A type copper ion is coupled to four D type copper ions. Two are at 8.779 Å, connected by a bridge of 10 diamagnetic atoms including one H-bond between the oxygen $O3^A$ and the nitrogen $N3^D$ ($d(O3^A-N3D) = 2.995$ Å), and total bond length d'= 16.690 Å. Two at d = 11.816 Å are connected by a path of nine atoms with total bond distance d' = 14.688 Å, including 2 H-bonds (Figure 7a,b). This structural information will be used to discuss the magnetic parameters.



Figure 5. Chemical path connecting copper neighbors types A and C rotated 180° around *b*, involving six σ -bonds and a π -cation interaction between the copper ion and the indole ring of the neighbor molecule. (a) Molecular drawing as obtained from the structural data in ref 24. (b) Diagram showing the six atoms in the path. Distances between the copper ions (*d*) and distances measured along the bonds (*d'*) are indicated.

4. EPR Results and Discussion

4.1. g Tensor and the Collapse of the Resonances. In the absence of exchange couplings between Cu ions, the EPR spectra of Cu^{II}Gly-Trp are described by the Zeeman contribution to the spin-Hamiltonian:

$$\mathbf{H}_{\mathrm{Z}} = \mu_{\mathrm{B}} \sum_{\alpha = \mathrm{A}, \mathrm{B}, \mathrm{C}, \mathrm{D}} \mathbf{S}_{\alpha} \cdot \mathbf{g}_{\alpha} \cdot \mathbf{B}$$
(1)

where S_{α} and g_{α} are the effective spin and the molecular **g** tensor corresponding to Cu(II) in site α , $\mathbf{B} = B\mathbf{h}$ is the applied magnetic field, and $\mu_{\rm B}$ is the Bohr magneton. $\mathbf{g}_{\rm A}$, $\mathbf{g}_{\rm B}$, $\mathbf{g}_{\rm C}$, and $\mathbf{g}_{\rm D}$ are related by the symmetry operations of the space group $P2_12_12_1$. Thus, $\mathbf{g}_{\rm A}$ is equal to $\mathbf{g}_{\rm B}$ (and $\mathbf{g}_{\rm C} = \mathbf{g}_{\rm D}$) in the *ab* plane, $\mathbf{g}_{\rm A}$ is equal to $\mathbf{g}_{\rm C}$ (and $\mathbf{g}_{\rm B} = \mathbf{g}_{\rm D}$) in the *ca* plane, and $\mathbf{g}_{\rm A}$ is equal to $\mathbf{g}_{\rm D}$ (and $\mathbf{g}_{\rm B}$ to $\mathbf{g}_{\rm C}$) in the *cb* plane. One expects to observe four EPR copper signals corresponding to the A, B, C, and D rotated copper sites in the unit cell, for an arbitrary orientation of the magnetic field in a single-crystal sample, two resonances when the magnetic field **B** is within one of the crystal planes, and one when the magnetic field is oriented along the crystal axes *a*, *b*, and *c*. This situation changes when the copper ions at different sites i,j are coupled by exchange interactions

$$\mathbf{H}_{\text{ex}}(\mathbf{i},\mathbf{j}) = -J_{\mathbf{ij}}\mathbf{S}_{\mathbf{i}}\cdot\mathbf{S}_{\mathbf{j}} \qquad \mathbf{i} \neq \mathbf{j}, \qquad \mathbf{i}, \mathbf{j} = \mathbf{A}, \mathbf{B}, \mathbf{C}, \mathbf{D} \quad (2)$$

When $|J_{ij}|$, is larger than the difference between the Zeeman energies, $\Delta E_z = |(\mathbf{g}_i - \mathbf{g}_j)\mu_B \mathbf{B}|$, the resonances corresponding to sites i and j collapse to a single one, reaching the "collapsed regime". The process of collapse and the behavior of the positions and the widths of the resonances due to this exchange narrowing process were described by Anderson.^{20,26} In previous papers we introduced procedures to evaluate the exchange coupling from the EPR spectra.^{15,19,23}

The data in Figure 1a,b indicate a collapsed resonance regime for any direction of the applied magnetic field in the *ca* and *cb* crystal planes. In that case the magnetic field position of the signal is the average of the positions of the signals that would correspond to the four sites. In the *ab* plane both the collapsed

TABLE 1: Values of the Components of the Crystal g^2 Tensor of Cu^{II}Gly-Trp Calculated from a Least-Squares Fitting of the Function $g^2(\theta,\phi) =$ hggh to the EPR Data at 9.5 and 35 GHz in Figure 1^{*a*}

	9.5 GHz	35 GHz
$(g^2)_{xx}$	4.2904(1)	4.2641(1)
$(g^2)_{yy}$	4.7022(3)	4.7133(1)
$(g^2)_{zz}$	4.3350(4)	4.2991(1)
$(g^2)_{xy}$	$\pm 0.1769(1)$	$\pm 0.1878(5)$
$(g^2)_{zx}$	0.00002(2)	0.00006(2)
$(g^2)_{zy}$	0.00003(2)	0.00005(2)
$(g^2)_1$	4.2248(3)	4.1959(3)
$(g^2)_2$	4.7678(3)	4.7815(3)
$(g^2)_3$	4.3350(1)	4.2991(1)
v_1	$(0.94, \pm 0.34, 0)$	$(0.94, \pm 0.34, 0)$
v_2	$(\pm 0.34, 0.94, 0)$	$(\pm 0.34, 0.94, 0)$
v_3	(0,0,1)	(0,0,1)
$g_{ }$	2.2086(7)	2.2102(7)
g_{\perp}	2.0554(3)	2.0484(3)
$\bar{2}\lambda$, deg	131.5	134.5

^{*a*}(g^2)₁, (g^2)₂, (g^2)₃ and v_1 , v_2 , v_3 are, respectively, the eigenvalues and eigenvectors of this tensor. The principal molecular *g*-values (g_{\parallel} , g_{\perp}) obtained as described in the text are included. 2λ is the angle between the normals to the coordination planes of the copper of copper ions in sites A and C. They should be compared with the crystallographic value $2\lambda = 131.5^{\circ}$. The number in parentheses is the uncertainty of the last significant digit.

and the noncollapsed resonance regimes are observed, with two lines collapsing into a single one for directions near the crystal axes. The positions of the two signals are the positions of the resonances corresponding to sites A and B, and C and D. Table 1 contains the values of the components of the crystal $\mathbf{g} \cdot \mathbf{g} = \mathbf{g}^2$ tensor obtained by a least-squares fit of the function $g^2(\theta,\varphi) =$ **hggh** to the experimental results in Figure 1a,b. The double signs consider the two resonances observed in the *ab* plane. The values of these components were used to obtain the solid lines shown in Figure 1a,b.

To calculate the components of the four symmetry-related molecular **g** tensors \mathbf{g}_A , \mathbf{g}_B , \mathbf{g}_C , and \mathbf{g}_D from the crystal \mathbf{g}^2 tensor, we assumed they have axial symmetry, a valid approximation because the copper coordination is close to square planar, and used the procedure described in refs 27 and 28. The calculated parallel and perpendicular molecular *g* values, g_{\parallel} and g_{\perp} , and the angle 2λ between the normals to the equatorial planes of the copper ions are included in Table 1. The differences between the \mathbf{g}^2 tensors calculated at 9.5 and 35 GHz may be attributed to small nonsecular contributions.²⁹ The results for 2λ in Table 1 are in good agreement with the value $2\lambda = 131.5^{\circ}$ obtained from the crystallographic data, indicating the validity of the axial symmetry approximation.

The principal values of the molecular **g** tensor, g_{\parallel} and g_{\perp} , given in Table 1, indicate that the main contribution to the ground-state wave function is a $d(x^2-y^2)$ orbital, as observed before in other copper compounds with amino acids and peptides.^{13,30–32}

The single line observed in the *ca* plane indicates that at least one of the couplings J_{AB} or J_{AD} is strong enough to collapse the resonances corresponding to these sites. Also, the single line observed in the *cb* plane indicates that at least one of the couplings J_{AB} or J_{AC} is strong enough to collapse the resonances corresponding to these sites. Meanwhile, the two resonances observed in most directions of the *ab* plane indicate weaker values of both J_{AC} and J_{AD} , which are not capable of collapsing the resonances of sites A and C, and A and D, in most directions within this plane. These three conditions are satisfied when the interactions between neighbor copper sites A and C, and A and D, are smaller, and the interaction between copper sites A and B (related by a C₂ rotation around the *c* axis) is larger than the difference between their Zeeman energies.

4.2. Exchange Interactions. The magnitudes of the exchange interactions are calculated from the EPR line width data using a model based on the theories describing the exchange narrowing phenomena.^{20,21} As described in ref 19, the magnetic system is described by a spin Hamiltonian obtained by adding the exchange interactions to the Zeeman interaction in eq 1. \mathbf{H}_Z is divided in two terms; the first is the average of the contribution of the collapsed sites, \mathbf{H}_{Zo} , and the second (residual Zeeman energies for these sites. The exchange interactions between a pair of sites commute with \mathbf{H}_{Zo} , but not with \mathbf{H}_{Zr} , which is time modulated and averaged to zero. However, the residual Zeeman term \mathbf{H}_{Zr} still contributes to the peak-to-peak line width,¹⁹

$$\Delta B_{\rm pp}(\theta,\phi) = \sqrt{\frac{2\pi}{3}} \frac{\omega_0^2 \hbar}{g\mu_{\rm B}} \sum_{u=1,3} \frac{1}{\omega_{\rm ex}^{(u)}} \frac{(\mathbf{h} \cdot \mathbf{g} \cdot \mathbf{G}_u \cdot \mathbf{h})^2}{g^4(\theta,\phi)}$$
(3)

where $\omega_{\text{ex}}^{(u)}$ are the exchange frequencies between one copper ion and other nonequivalent neighbor copper ion. When \mathbf{H}_{Zr} is the main contribution to $\Delta B_{\text{pp}}(\theta, j)$, eq 3 shows that the line width depends quadratically on the microwave frequency ω_0 . This explain the dramatic differences shown in Figure 2a,b when one goes from 9.5 to 35 GHz and supports the role of \mathbf{H}_{Zr} as the main broadening mechanism.

Each term $(\mathbf{h} \cdot \mathbf{g} \cdot \mathbf{G}_u \cdot \mathbf{h})^2$ in eq 3 is proportional to one nondiagonal matrix element of g_A , g_B , g_C , and g_D in the crystal axes system as^{15,19}

$$(\mathbf{h} \cdot \mathbf{g} \cdot \mathbf{G}_1 \cdot \mathbf{h})^2 / \mathbf{g}^2(\theta, \phi) \approx g_{yz}^2 \sin^2 \theta \cos^2 \theta$$

(in the *cb* plane) (4a)

$$(\mathbf{h} \cdot \mathbf{g} \cdot \mathbf{G}_2 \cdot \mathbf{h})^2 / g^2(\theta, \phi) \approx g_{xz}^2 \sin^2 \theta \cos^2 \theta$$

(in the *ca* plane) (4b)

$$(\mathbf{h} \cdot \mathbf{g} \cdot \mathbf{G}_3 \cdot \mathbf{h})^2 / g^2(\theta, \phi) \approx g_{xy}^2 \sin^2 \varphi \cos^2 \varphi$$

(in the *ab* plane) (4c)

The relationship between the exchange frequencies $\omega_{ex}^{(u)}$ and the magnitude of the exchange couplings $J^{(\alpha,\beta)}$ for a lattice with four rotated ions can be expressed as¹⁹

$$(\omega_{\rm ex}^{(1)})^2 = \frac{1}{\hbar^2} [4(J^{(A,B)})^2 + 2(J^{(A,C)})^2]$$
(5a)

$$(\omega_{\rm ex}^{(2)})^2 = \frac{1}{\hbar^2} [4(J^{(A,B)})^2 + 4(J^{(A,D)})^2]$$
(5b)

$$(\omega_{\rm ex}^{(3)})^2 = \frac{1}{\hbar^2} [2(J^{(\rm A,C)})^2 + 4(J^{(\rm A,D)})^2]$$
(5c)

where the numbers of equivalent neighboring molecules to each Cu(II) ion are already considered (see Crystal Structure section).

The EPR measurements at different frequencies allow us to calculate three exchange frequencies $\omega_{ex}^{(u)}$ associated with the terms of \mathbf{H}_{Zr} .¹⁹ To do that, we calculated the differences $(\Delta B_{pp}-(\theta,\varphi))_Q - (\Delta B_{pp}(\theta,\varphi))_X$ between the line widths measured at 35 and 9.5 GHz in the *ca* and *cb* planes (Figure 2a,b), and in the collapsed region in the *ab* plane (Figure 3). Using eq 3, we obtain

$$(\Delta B_{\rm pp}(\theta,\phi)_{\rm Q}) - (\Delta B_{\rm pp}(\theta,\phi)_{\rm X}) = \sqrt{\frac{2\pi}{3} \frac{(\omega_{\rm Q}^2 - \omega_{\rm X}^2)\hbar}{g\mu_{\rm B}\omega_{\rm ex}^{(u)}}} \frac{(\mathbf{h}\cdot\mathbf{g}\cdot\mathbf{G}_u\cdot\mathbf{h})^2}{g^4(\theta,\phi)}$$
(6)

Using eqs 4–6, the values of the nondiagonal components of the molecular **g** tensors, $g_{xy} = 0.044$, $g_{xz} = -0.0196$, and $g_{zy} = -0.054$, calculated from the data in Figure 1, and the line width results in Figures 2 and 3, we calculated $\hbar \omega_{ex}^{(1)}/k_B = 25 \pm 1$ mK from the data in the *cb* plane, $\hbar \omega_{ex}^{(2)}/k_B = 25 \pm 1$ mK from the data in the *cb* plane, $\hbar \omega_{ex}^{(2)}/k_B = 9 \pm 2$ mK from the data in the *cb* plane, and $\hbar \omega_{ex}^{(3)}/k_B = 9 \pm 2$ mK from the data in the *ab* plane. Using eqs 4–6, we solved the system for the magnitudes of *J*, obtaining $|J^{(A,B)}/k_B| = 12(3)$ mK, $|J^{(A,C)}/k_B| = 5(2)$ mK, and $|J^{(A,D)}/k_B| = 3(1)$ mK. The EPR method does not allow obtaining the sign of the interaction.

5. Discussion and Conclusion

First it is interesting to compare the values obtained for the exchange couplings J_{AB} , J_{AC} , and J_{AD} with the magnitude of the magnetic dipolar coupling between these ions. Considering the distances between Cu ions calculated from the structural data, dipolar couplings should be smaller than $E_d/k_B \sim 1.5$ mK, not much smaller than the value obtained for the coupling between A and D type copper ions. However, the angular dependence of the line width observed in the *ab* plane, in the collapsed resonances region (i.e., close to the crystal axes), is typical of an exchange narrowing process (eq 3), and cannot be explained by dipolar couplings. It is worth clarifying that the dipolar interactions, which may have magnitudes similar to those of the exchange interactions considered in Cu^{II}Gly-Trp, are time modulated by these exchange interactions, and their effect on the EPR spectra is thus reduced.^{20,21}

No information is obtained from our experiments for the exchange interactions between equally oriented copper ions (A–A interaction). We have shown above that the EPR data provide values for the exchange couplings between a copper ion type A and the neighbor copper ions type B, C, and D. Those values along with the distances involved in each path are summarized in Table 2. In the interpretation of our experimental results we assumed that the exchange coupling between coppers type A and B through the different paths shown



 $d = 8.467 \text{ Å}, \quad d' = 11.766 \text{ Å}$

Figure 6. Chemical path connecting a copper ion type A with two copper neighbors type B rotated 180° around *c*, involving seven diamagnetic atoms and including two H-bonds. (a) Molecular drawing as obtained from the structural data in ref 24. (b) Diagram showing the six atoms in the path. Distances between the copper ions (*d*) and distances measured along the bond (*d'*) are indicated.



Figure 7. Chemical paths connecting copper neighbors types A and D rotated 180° around *a*, involving seven diamagnetic atoms, including one H-bond. (a) Molecular drawing as obtained from the structural data in ref 24. (b) Diagram showing the six atoms in the path. Distances between the copper ions (*d*) and distances measured along the bond (*d'*) are indicated.

in Figure 6a,b are the same. This approximation is well supported by the high similarity of these paths. We also assumed that the interactions through the two different paths connecting

A type Cu ions to four D type Cu ions (see Figure 7a,b) are equal. One path contains one H-bond, and the other contains two. However, the last one is shorter and contains one atom

 TABLE 2: Magneto-Structural Correlation for the Several

 Exchange Interactions Described in the Text

path	distance <i>d</i> between Cu ions (Å)	bond distance $d'(\text{\AA})$	$ J/k_{\rm B} $ (mK)	shown in
AB	8.377, 8.467	11.900, 11.766	12(3)	Figure 6
AC	7.207	12.354	5(2)	Figure 5
AD	8.779, 11.816	16.690, 14.688	3(1)	Figure 7

less than the first. We have no further data to support this assumption. If the contributions between these paths were different, they could change the calculated magnitude of J_{AD} by a factor of up to $\sqrt{2}$. In the case of the paths between A and C copper neighbors, they are symmetry related and no approximation is done in our calculation.

The largest exchange coupling between Cu(II) ions observed in Cu^{II}Gly-Trp, $|J_{AB}|/k_B = 12(3)$ mK, is transmitted by a path involving four σ -bonds and two H-bonds that involve a nonbonded water molecule (O5^W or O6^W). Our result could be compared with the value $J/k_B = -3.8(1)$ mK reported recently^{16,33} between two semiquinone radicals Q_A⁻ and Q_B⁻ connected by a path containing six σ -bonds plus two H-bonds, in a bacterial photosynthetic reaction center protein. In this case the path length is 17.3 Å, longer than in Cu^{II}Gly-Trp. Considering the bond lengths for each case, one could argue that the relatively small value of J_{AB} reported in this work is a consequence of the presence of a nonbonded water molecule in the exchange path.

The result found here $|J_{AC}/k_B| = 5(2)$ mK for the coupling transmitted through the path containing six σ -bonds and the cation- π contact is smaller than that reported before for a similar path in Cu^{II}Trp-Gly.¹⁵ In the case of Cu^{II}Trp-Gly a Cu-(II) ion is coupled by cation- π interactions to indole rings on both apical positions, but this argument may not be enough to explain the difference in the magnitude of the coupling. Theoretical analyses of the exchange coupling in the case of bridges containing cation- π contacts do not exist yet. They could clarify the role of the detailed geometry of the bonds and explain the observed differences.

In conclusion, we have determined the exchange interactions connecting Cu(II) ions through paths longer than 12 Å in the Cu(II) complex of the peptide Gly-Trp. The evaluation of the magnitudes of very small interactions can be achieved by means of their effects on room temperature EPR spectra. The use of model compounds allows one to obtain information on biologically relevant bonds, which can be conveniently separated from others by choosing appropriate amino acids or small peptides. To our knowledge, this is the second example of a system where cation– π interactions were quantitatively determined.¹⁵ A model for the exchange interaction transmitted through cation– π contacts and a more detailed comparison with the previous results are still needed.

The magnitudes of the exchange couplings through long chemical paths obtained in this work are helpful to analyze their relation with the transmission coefficients for electron transfer between redox centers in proteins.^{16,17} The distance dependence agrees with general facts discussed before,¹⁷ but more experimental information is needed to make further progress in this direction.

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