Inorg. Chem. **2006**, *45*, 2942–2947

Inorganic Chemistry
Article

Weak Exchange Interaction Supported by a Biologically Relevant Long Chemical Bridge in a Cu–Peptide Model Compound

**Ernanni D. Vieira,[†] Nieves M. C. Casado,[§] Gianella Facchin,[‡] Maria H. Torre,[‡]
Antonio J. Costa-Filho,[†] and Rafael Calvo*,[§]**

*Grupo de Biofísica Molecular Sergio Mascarenhas, 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, Departamento de Física, Facultad de Bioquímica y Ciencias Biológicas,
Universidad Nacional del Litoral and INTEC (CONICET-UNL), Casilla 530,
3000 Santa Fe, Argentina, and Química Inorgánica, DEC, Facultad de Química,
Universidad de la República, Gral. Flores 2124, CC 1157 Montevideo, Uruguay*

Received November 11, 2005

The copper complex of the dipeptide L-alanyl-L-phenylalanine, catena-(L-alaninate-L-phenylalaninate-copper(II) monohydrate), identified as Cu(II)Ala-Phe, provides a convenient system to study a weak exchange interaction between unpaired spins transmitted through a biologically relevant long chemical bridge (18.34 Å). In this complex, the copper ions are arranged in two symmetry-related anisotropic layers parallel to the *ab* plane at 13.17 Å, separated by a double layer of water molecules. The equatorial–equatorial bridge considered as the most relevant path for exchange interactions between copper ions in neighbor layers contains 11 diamagnetic atoms (including three hydrogens), with two covalent amidate bridges plus three weak and moderate H bonds that go across the water layer. This interaction was studied using electron paramagnetic resonance in single-crystal samples, at 9.5 and 34.5 GHz. The measured magnitude of the interlayer interaction, $|J_3|/k_B = 1.7(2) \times 10^{-3}$ K, is discussed in terms of values obtained for similar paths in other model compounds and in proteins. These results in model systems provide information that may be important in understanding biological functions at the molecular level.

Introduction

Long and weak chemical bonds are important in biological molecules for electron transfer, molecular recognition, and other functions.[1] They also contribute to the generation of the supramolecular structure of proteins. When these long chemical paths connect unpaired spins S_i, they sustain weak exchange interactions (defined as[2] $\mathscr{H}_{ex} = -JS_iS_j$), whose magnitudes can be measured using electron paramagnetic resonance (EPR).[3] Exploiting the phenomena of exchange narrowing and collapse,[4] EPR experiments in single crystal samples, usually performed at room temperature, allow the selective evaluation of weak isotropic exchange interactions in the presence of much stronger couplings.[5,6] The magnitudes of these exchange interactions provide information about the electronic structure of the diamagnetic molecular paths bridging the unpaired spins[2] that is difficult to obtain from other sources. When analyzed in terms of the crystal structure, they allow a characterization of the properties of the long paths. As a possible application, the exchange coupling parameter J is related to the matrix element for electron-transfer processes along the path.[7−12] Thus, it is useful to study exchange interactions in model compounds and extrapolate these findings to biological molecules.

---

* To whom correspondence should be addressed. Fax: +54-342-460-8200. E-mail: calvo@fbcb.unl.edu.ar.

[†] Universidade de São Paulo.

[‡] Universidad de la República.

[§] Universidad Nacional del Litoral.

(1) Lippard, S. J.; Berg, J. M. *Principles of Bioinorganic Chemistry*; University Science Books: Mill Valley, CA, 1994.

(2) Kahn, O. *Molecular Magnetism*; VCH: New York, 1993.

(3) Bencini, A.; Gatteschi, D. *Electron Paramagnetic Resonance of Exchange Coupled Systems*; Springer: Berlin, 1990.

(4) (a) Anderson, P. W. *J. Phys. Soc. Jpn.* **1954**, *9*, 316−339. (b) Abragam, A. *The Principles of Nuclear Magnetism*; Oxford University Press: London, 1961; Chapter X.

(5) Levstein, P. R.; Calvo, R. *Inorg. Chem.* **1991**, *29*, 1581−1583.

(6) Passeggi, M. C. G.; Calvo, R. *J. Magn. Reson., Ser. A* **1995**, *114*, 1−11.

(7) (a) Okamura, M. Y.; Isaacson, R. A.; Feher, G. *Biochim. Biophys. Acta* **1979**, *546*, 394−417. (b) Okamura, M. Y.; Fredkin, D. R.; Isaacson, R. A.; Feher, G. *Quantum Mechanical Tunneling in Biological Systems*; Chance, B., DeVault, D. C., Frauenfelder, H., Marcus, R. A., Suttin, N., Eds.; Academic Press: New York, 1979; pp 729−743.

10.1021/ic051957b CCC: $33.50

In previous works, we reported EPR and magnetic measurements of weak exchange interactions in complexes of metal ions with amino acids or small peptides.[13−15] In this work, we evaluate an extremely weak exchange interaction supported by a long chemical path of 18.34 Å bridging copper ions in the copper complex of the peptide L-alanyl-L-phenyalanine. This path includes a sequence of strong covalent bonds plus two moderate and one weak hydrogen bond. The results are compared with the exchange interaction transmitted by similar paths containing σ bonds and H bonds in biomolecules and other model compounds.

Materials and Methods

Room-temperature EPR measurements were performed with an ER-200 Bruker spectrometer operating at 9.5 GHz (X band) and a Varian E109 operating at 34.5 GHz (Q band).

Single crystals of catena-(L-alaninate-L-phenylalaninate-copper-(II) monohydrate), $(C_{12}H_{14}CuN_2O_3)_n{\cdot}nH_2O$, identified as Cu(II)-Ala-Phe, were obtained as described previously.[16] For the EPR experiments, we used blue crystals elongated along the *a* axis, which were glued to cleaved KCl cubic sample holders with the *a*, *b*, and *c* crystal axes parallel to the *x*, *y*, and *z* axes of the holder, defining the laboratory reference frame. These holders were positioned on a pedestal with a horizontal plane inside the cavity, and the angular variation of the spectra was measured as a function of the orientation of the applied magnetic field $\mathbf{B} = Bh$, where $h = (\sin\theta\cos\phi, \sin\theta\sin\phi, \cos\theta)$, in three mutually orthogonal crystal planes (*ab*, *bc*, and *ac*).

Results and Analysis

EPR Results. A single anisotropic resonance without hyperfine structure was observed for all orientations of the magnetic field in the *ab* and *bc* crystal planes. Two resonances were observed for most orientations of the applied magnetic field **B** within the *ac* plane. These two resonances collapse to a single one in the vicinity of the *a* and *c* axes in the *ac* plane. The positions and widths of the resonances were obtained by fitting a Lorentzian derivative function, or the sum of two Lorentzian derivative functions, to the observed spectra. Parts a and b of Figure 1 display the angular variation of $g^2(\theta,\phi)$ measured at 34.5 and 9.5 GHz in the three crystal planes. Parts a and b of Figure 2 display the angular variation of the peak-to-peak line width $\Delta B_{pp}(\theta,\phi)$ obtained at these frequencies in the same experiments. Because the angular variation of the width of the single resonance observed in a narrow angular range close to the *a* and *c* axes in the *ac* plane is of great importance for our purpose, this was measured at both microwave frequencies in accurately oriented single crystals with closely spaced angular intervals (≤1°). The values of $g^2(\theta,\phi)$ given in Figure 1 were used to calculate the squared **g** tensors corresponding to coppers in each layer (see discussion below) using a least-squares procedure. Their components are included in Table 1.

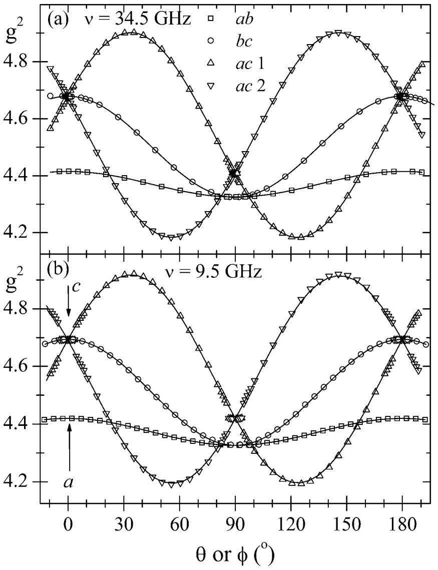

Figure 1. Experimental values of the $\mathbf{g}^2$ tensor for the resonance lines observed for Cu(II)Ala-Phe in the three crystallographic planes at (a) 34.5 and (b) 9.5 GHz. The solid lines were obtained with the parameters given in Table 1.

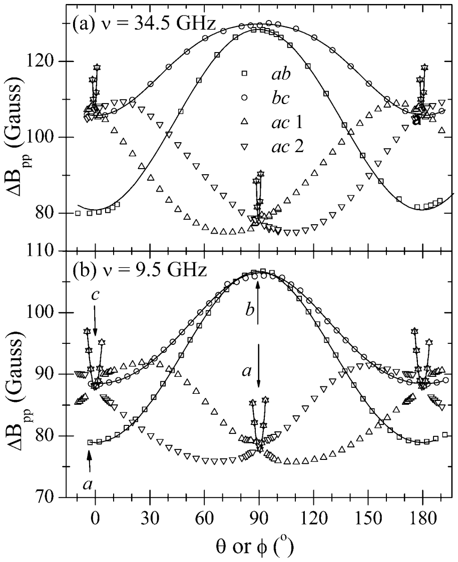

Figure 2. Values of the peak-to-peak line width (ΔB_{pp}) of the resonance lines observed for Cu(II)Ala-Phe in the three crystallographic planes at (a) 34.5 and (b) 9.5 GHz. The solid lines in the angular ranges close to the *a* and *c* axes in the *ac* plane were obtained using eq 4. The solid lines covering the full angular range in the *ab* and *bc* planes in parts a and b are added to help visualization.

Crystal Structure and Exchange Pathways in Cu(II)-Ala-Phe. The crystal structure of Cu(II)Ala-Phe is orthorhombic, space group $P2_12_12_1$, with lattice parameters $a =$

---

(8) De Vault, D. C. *Quantum Mechanical Tunneling in Biological Systems*; Cambridge University Press: London, 1984; pp 118−121.

(9) Bominaar, E. L.; Achim, C.; Borshch, S. A.; Girerd, J. J.; Münck, E. *Inorg. Chem.* **1997**, *36*, 3689−3701.

(10) Calvo, R.; Abresch, E. C.; Bittl, R.; Feher, G.; Hofbauer, W.; Isaacson, R. A.; Lubitz, W.; Okamura, M. Y.; Paddock, M. L. *J. Am. Chem. Soc.* **2000**, *122*, 7327−7341.

(11) Chen, P.; Solomon E. I. *Proc. Natl. Acad. Sciences U.S.A.* **2004**, *101*, 13105−13110.

(12) Weiss, E. A.; Wasielewski, M. R.; Ratner, M. A. *J. Chem. Phys.* **2005**, *123*, 064504.

(13) Costa-Filho, A. J.; Nascimento, O. R.; Ghivelder, L.; Calvo, R. *J. Phys. Chem. B* **2001**, *105*, 5039−5047.

(14) Costa-Filho, A. J.; Nascimento, O. R.; Calvo, R. *J. Phys. Chem. B* **2004**, *108*, 9549−9555.

(15) Santana, R. C.; Cunha, R. O.; Carvalho, J. F.; Vencato, I.; Calvo, R. *J. Inorg. Biochem.* **2005**, *99*, 415−423.

(16) Facchin, G.; Torre, M. H.; Kremer, E.; Piro, O. E.; Castellano, E. E.; Baran, E. J. *Z. Naturforsch.* **2000**, *55*, 1157−1162.

Table 1. Components of the $\mathbf{g}^2$ Tensors Obtained by Least Squares Fitting of the Function $g^2(\theta,\phi) = \mathbf{h}\cdot\mathbf{g}\cdot\mathbf{g}\cdot\mathbf{h}$ to the Experimental Data in Figure 1[a]

	9.5 GHz	34.5 GHz
$g_{xx}{}^2$	4.419(2)	4.409(1)
$g_{yy}{}^2$	4.3265(7)	4.3219
$g_{zz}{}^2$	4.693(7)	4.6769(8)
$g_{xy}{}^2$	0.0000(7)	0.0000(1)
$g_{zx}{}^2$	±0.335(4)	±0.333(1)
$g_{zy}{}^2$	0.0000(7)	0.0000(2)
$(g^2)_1$	4.194(4)	4.184(1)
$(g^2)_2$	4.3265(7)	4.3219(5)
$(g^2)_3$	4.918(4)	4.902(1)
$g_{\parallel}$	2.245(2)	2.2450(7)
$g_{\perp}$	2.0481(9)	2.0454(3)
$\lvert J_3\rvert/k_B$	1.5(2) $\times 10^{-3}$ K	1.8(2) $\times 10^{-3}$ K

[a] The double signs correspond to the resonances assigned to layers I and II, which are coincident in the planes *ab* and *bc* but have different *g* factors in the *ac* plane. The eigenvalues, $(g^2)_1$, $(g^2)_2$, and $(g^2)_3$, of these tensors are included. $g_\perp$ and $g_\parallel$ were calculated assuming axial symmetry for the Cu(II) molecular **g** tensor. The values of the exchange interaction between copper ions in neighbor layers obtained at each microwave frequency are included.

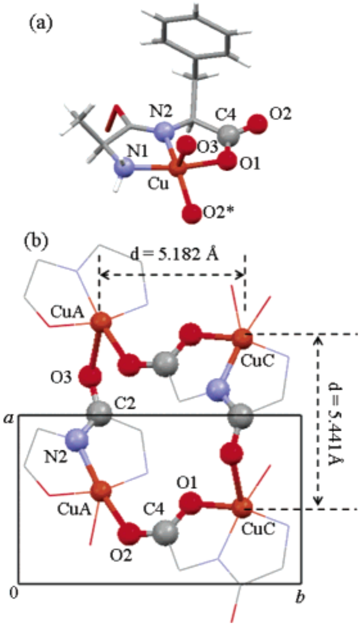

Figure 3. (a) Molecular structure of Cu(II)Ala-Phe according to ref 16. (b) Copper layer parallel to the *ab* plane. The carboxylate and amidate bridges connecting the copper ions are emphasized.

5.441(2) Å, *b* = 9.0578(10) Å, and *c* = 26.338(7) Å and four molecules (labeled A, B, C, and D) per unit cell (Z = 4).[16] Molecules B, C, and D are obtained from molecule A by 180° rotations around the *c*, *b*, and *a* axes, respectively, plus a translation.[17] They are chemically equal but magnetically nonequivalent in the presence of an applied magnetic field. The copper ion is at the center of a square-based pyramid (Figure 3a), equatorially coordinated to a dipeptide molecule acting as a tridentate ligand through the nitrogen atom of the amide group (N2), the nitrogen atom of the terminal NH_2 group (N1), and one of the oxygen atoms of the carboxylate group (O1). The fourth equatorial ligand is the other carboxylate oxygen atom (O2) from a neighboring dipeptide molecule. The apical ligand is the carbonyl oxygen atom (O3) of a third dipeptide molecule, obtained by a unit cell translation along the short *a* axis.

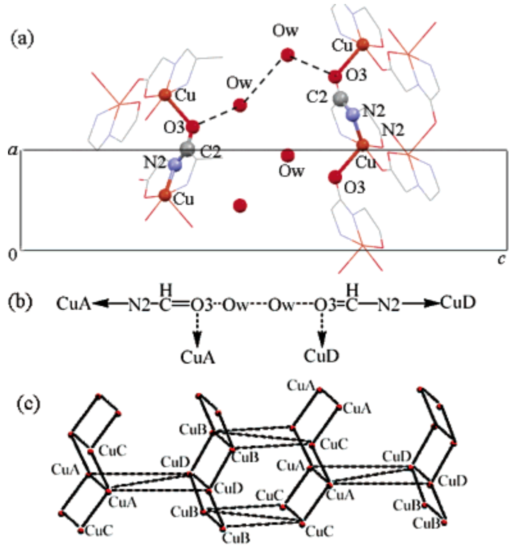

Figure 4. (a) Two neighbor copper layers separated by a water layer are displayed. The Ow in the figures are defined as O4 in ref 16. The chemical bridges connecting copper ions are emphasized. (b) Scheme of the interlayer bridging system connecting copper ions. (c) Exchange interaction network in Cu(II)Ala-Phe.

The strongest bonds between neighbor copper ions in Cu(II)Ala-Phe are equatorial−equatorial syn−anti carboxylate bridges connecting copper atoms in molecule types A and C (and types B and D) with $d[\mathrm{Cu^A{-}Cu^C}] = 5.18$ Å and a distance $d' = 6.47$ Å measured along the bonds (Figure 3b), giving rise to copper chains along the crystal *b* axis. In addition, a copper ion is connected by equatorial−apical amidate bridges (total bonds length $d' = 6.79$ Å) to two copper neighbors of the same type, separated by one lattice parameter along the *a* axis ($d[\mathrm{Cu^A{-}Cu^A}] = 5.44$ Å; Figure 3b). This gives rise to alternate anisotropic magnetic layers I and II, parallel to the *ab* plane, containing copper atoms A and C (layer I) and copper atoms B and D (layer II). Layer I is displayed in Figure 3b, and the distance between layers I and II is $c/2 = 13.17$ Å. Between them there is a layer of water molecules (Figure 4a). The strongest intralayer exchange coupling, called J_1, is supported by the equatorial−equatorial syn−anti carboxylate bond O1−C4−O2 between neighbor coppers in sites A and C in layer I (or B and D in layer II). Within a layer, the equatorial−apical amidate bonds N2−C2−O3 between neighbor coppers of the same type sustain an interaction with a magnitude called J_2 that is expected to be weaker than J_1 because it involves the Cu−O3 apical contact to copper.

The contacts between $\mathrm{Cu^A}$ and $\mathrm{Cu^D}$ (or $\mathrm{Cu^B}$ and $\mathrm{Cu^C}$) ions in neighboring copper layers ($d[\mathrm{Cu^A{-}Cu^D}] = 11.07$ Å) are supported by a bridging system shown in Figure 4a and in the scheme of Figure 4b. It contains a central portion involving a weak hydrogen bond between the oxygen atoms of the nonbonded water molecules Ow and Ow′ with $d[\mathrm{Ow{-}Ow'}] = 3.76$ Å. It also contains two identical and moderate hydrogen bonds between oxygens Ow and the carbonyl

---

(17) *International Tables for X-ray Crystallography. Space-Group Symmetry*; Hahn, T., Ed.; Reidel: Dordrecht, The Netherlands, 1987; Vol. A.

oxygen O3 of the amide groups (d[O3−Ow] = 2.82 Å). The O3 atoms are connected equatorially to other copper types A and D through the intralayer amidate bonds described above (see Figure 4a,b). Besides that, the O3 oxygens are apically connected to copper types A and D, giving rise to an interlayer apical−apical bridge between copper atoms. Thus, the interlayer bridging system between coppers A and D at 11.07 Å in neighbor layers is composed of two bridges through the water layer: (a) an equatorial-to-equatorial bridge containing 11 diamagnetic atoms (including the three hydrogens in the hydrogen bonds), with a distance along this path $d' = 18.34$ Å (Figure 4a and scheme in Figure 4b), and (b) an apical−apical path containing seven diamagnetic atoms (including the three hydrogen atoms in the hydrogen bonds), with a distance along this path $d' = 14.05$ Å (Figure 4a and scheme in Figure 4b). Because the apical Cu−O3 segment of bridge type b is a weak contact, one may assume that bridge a supports the strongest contribution to the exchange interaction, J_3, between copper ions in neighbor layers. The connections between coppers type A and type B in neighbor layers are longer and less important. Figure 4c describes the intralayer and interlayer exchange networks acting in Cu(II)Ala-Phe. The purpose of this investigation is to evaluate, from a single-crystal EPR study of Cu(II)-Ala-Phe, the exchange coupling J_3 transmitted along the chemical path going across the water layer described in Figure 4a and b and to correlate its value to the structure of the path. Our result is compared to similar measurements in other model systems and in a protein.

Analysis of the EPR Results. The EPR spectra of Cu(II)Ala-Phe can be described by a spin Hamiltonian containing Zeeman ($\mathscr{H}_z$) and exchange ($\mathscr{H}_{ex}$) terms, as described in ref 3 (Chapter 2). Dipolar interactions are not considered here (see text further on for more details). The described layered structure of the compound allows writing the Hamiltonian as

$$\mathscr{H} = \mathscr{H}_I + \mathscr{H}_{II} + \mathscr{H}' \qquad (1)$$

where $\mathscr{H}_I$ and $\mathscr{H}_{II}$ contain the contributions within layers I and II, respectively, and $\mathscr{H}'$ contains the relevant interlayer exchange interactions. Thus

$$\mathscr{H}_I = \mu_B \sum_{i=1}^{N} (\mathbf{B}\cdot\mathbf{g_A} S_{iA} + \mathbf{B}\cdot\mathbf{g_C} S_{iC}) - \sum_{i>j} J_1 S_{iA} S_{jC} - \sum_{i>j} J_2 S_{iA} S_{jA} - \sum_{i>j} J_2 \cdot S_{iC} \cdot S_{jC} \qquad (2)$$

In eq 2, **B** is the applied magnetic field, μ_B is the Bohr magneton, the index i runs over the crystal cells, $\mathbf{g_A}$ and $\mathbf{g_C}$ are the molecular **g** tensors corresponding to copper ions in sites A and C, respectively, and S_{iA} and S_{iC} are the effective spins corresponding respectively to Cu(II) ions in sites A and C of cell i. Similar terms with sites A and C replaced by sites B and D, respectively, contribute to $\mathscr{H}_{II}$. The sums in the exchange contributions are only over nearest neighbor (i and j) pairs. Other intralayer interactions are assumed to be smaller and are neglected. At this point, one should notice that the two terms containing the exchange coupling parameter J_2 in eq 2 commute with the Zeeman terms, and do not contribute to the EPR spectrum.

The interlayer interaction $\mathscr{H}'$ can be written as

$$\mathscr{H}' = -\sum_{i>j} J_3 (S_{iA} S_{jD} + S_{iB} S_{jC}) \qquad (3)$$

where the sums are over nearest neighbors A−D or B−C Cu(II) pairs. The EPR spectra of the spin system described by the spin Hamiltonian of eqs 1−3 have been described before.[5,6] According to Anderson's theory of exchange narrowing and collapse in magnetic resonance,[4] we propose, for the present case, that the magnitude of the intralayer exchange interaction J_1 is large enough to collapse to one the two resonances which would be observed for the two rotated copper ions in each layer. One observes a resonance with **g** tensor $\mathbf{g_I} = {}^1/_2(\mathbf{g_A} + \mathbf{g_C})$ for layer I and a resonance with $\mathbf{g_{II}} = {}^1/_2(\mathbf{g_B} + \mathbf{g_D})$ for layer II. The corresponding $\mathbf{g}^2$ tensors, whose components are given in Table 1, are assigned to these copper layers. In the *ab* and *bc* planes, the resonances corresponding to layers I and II collapse to a single one by symmetry conditions ($\mathbf{g_I} = \mathbf{g_{II}}$ for the magnetic field in these planes). In the *ac* plane, where the g factors g_I and g_{II} are different, we observe two resonances except in narrow angular ranges around the axes, where the weak interlayer exchange interaction J_3 is capable of collapsing the resonances (i.e., $|J_3| > |g_I - g_{II}|\mu_B B$), as shown in Figures 1 and 2.

The molecular **g** tensors $\mathbf{g_A}$−$\mathbf{g_D}$ should reflect the approximate axial symmetry of the copper molecules. We estimated values of $g_{||}$ and $g_{\perp}$ for these molecular tensors assuming full axial symmetry and following a procedure described previously.[18] These values are included in Table 1; the calculation also provides the orientations of the axial directions of the molecular **g** tensors (eigenvectors corresponding to $g_{||}$, not included in Table 1) that agree within 3° with the values obtained from the structural information.[16] This supports the assignments given to the resonances. Because the EPR spectra show only one resonance for every orientation of **B** in the *ab* and *bc* planes at 34.5 and 9.5 GHz, one can set a lower limit $|J_1|/k_B > 0.5$ K for the magnitude of the intralayer exchange interaction.[19] This result explains the absence of a hyperfine structure, which is averaged out by the intralayer exchange interactions J_1 and J_2.

The magnitude J_3 of the interlayer exchange interactions was calculated from the values of the EPR line width observed in the *ac* plane, close to the *a* and *c* axes, where only one resonance is observed. Considering the exchange narrowing theory,[4] the peak-to-peak width of the resonances observed close to the *a* and *c* axes in the *ac* planes is given by[5,6]

$$\Delta B_{pp}(\theta,\phi) = \frac{\omega_0^{\,2}\hbar}{g\mu_B}\sqrt{\frac{2\pi}{3}}\,\frac{(\mathbf{h}\cdot\mathbf{g}\cdot\mathbf{G}\cdot\mathbf{h})^2}{g^4\omega_{ex}} + \Delta B_{pp}(0) \qquad (4)$$

$\Delta B_{pp}(0)$ is the residual line width observed when the

---

(18) Calvo, R.; Mesa, M. A. *Phys. Rev. B.* **1983**, *28*, 1244−1248.

Table 2. Exchange Couplings Supported by Long Superexchange Bridges Connecting Copper Ions in Copper Complexes[a]

	number of atoms	d(Cu−Cu) (Å)	d′(Cu−Cu) (Å)	H bonds	$\lvert J\rvert/k_B$ [10^{-3}K]	ref
Cu(D,L-proline)$_2$·2H$_2$O (**1**)	5	9.76	11.46	1 weak	48	21
CuBr$_2$(1-phenyl-3,5 dimethylpirazole)$_2$ (**2**)	5	8.89	11.15	1 weak	180	22
CuCl$_2$(1-phenyl-3,5 dimethylpirazole)$_2$ (**3**)	7	8.33	13.60	1 weak	9	22
aqua(glycyl-L-tryptophanate) copper(II) dihydrate (**4**)	7 (**a**)	8.38−8.47	11.90−11.77	2 mod	12	14
	10 (**b**)	8.78	16.69	1 mod	3	14
L-alaninate-L-phenylalaninate-copper(II) monohydrate (**5**)	11	11.07	18.34	2 mod	1.8	this work
copper(II)(L-arginine)$_2$ (SO$_4$)·(H$_2$O)$_6$ (**6**)	12	14.09	19.18	2 mod	9	15

[a] Included are the number of diamagnetic atoms in the bridge, the distance *d* between the copper ions, the length *d*′ measured along the bridge, the number of H bonds in the bridge, and the reported exchange interaction $|J|$.

magnetic field is oriented along the crystal axes; g is the average g factor of the copper ion, and ω_0 is the resonance frequency of the experiments. The tensors $\mathbf{g} = {}^1/_2(\mathbf{g_I} + \mathbf{g_{II}})$ and $\mathbf{G} = {}^1/_2(\mathbf{g_I} - \mathbf{g_{II}})$ are the average and half the difference, respectively, of the **g** tensors corresponding to the two layers. The tensor **G** is related to the g anisotropy of the Cu(II) ions. The tensors $\mathbf{g_I}$ and $\mathbf{g_{II}}$ are given in Table 1, and thus, **g** and **G** are known. Equation 4 is used to fit the line width data in Figure 2a and b near the crystal axes in the *ac* plane, where the resonances are collapsed, and to calculate the value of ω_{ex} from the data at each microwave frequency. The interlayer exchange interaction J_3 responsible for the observed collapse of the resonances is related to the exchange frequency ω_{ex} introduced in eq 4 by[5,6]

$$\omega_{ex}^2 = \frac{1}{2\hbar^2} 2J_3^{\,2} \tag{5}$$

The values of the exchange coupling parameter obtained from eq 5 are $|J_3|/k_B = 1.5(2) \times 10^{-3}$ K at 9.5 GHz and $|J_3|/k_B = 1.8(2) \times 10^{-3}$ K at 34.5 GHz. The sign of J_3 cannot be determined from the EPR data.

Discussion and Conclusions

Our EPR measurements in single crystals of Cu(II)Ala-Phe allowed the determination of the magnitude of the exchange interaction between Cu^A and Cu^D in neighbor layers [$|J_3|/k_B = 1.7(2) \times 10^{-3}$ K] transmitted through a long path (18.34 Å), which includes three hydrogen bonds between two water molecules and two amidate bridges. The dipolar and the anisotropic exchange interactions between coppers in the same layer are partially averaged out by the large intralayer exchange interactions,[4] and they contribute to the residual line width $\Delta B_{pp}(0)$ in eq 4 but not to the parabolic angular dependence observed around the *a* and *c* axes in the *ac* plane (Figure 2). The magnitudes of the dipolar interactions between the closest copper ions in neighbor layers are $\sim 1.0 \times 10^{-3}$ K, and they are averaged out by the much larger intralayer exchange interactions.[6,20]

Weak exchange interactions sustained by biologically relevant paths in model systems and in complex biological molecules have been reported previously. We can compare the value of $|J_3|$ for Cu(II)Ala-Phe with those observed for long bridges containing H bonds. In [Cu(D,L-proline)$_2$·2H$_2$O],[21] an exchange interaction with magnitude $|J|/k_B = 0.048$ K was observed between Cu atoms at 9.76 Å connected by an apical−equatorial bridge, Cu−O$_{ap}$···H−C−C−N$_{eq}$−Cu, with a total length of 11.46 Å and containing five diamagnetic atoms with a weak hydrogen bond. In the case of CuBr$_2$(1-phenyl-3,5dimethylpirazole)$_2$,[22] an exchange interaction $|J|/k_B = 0.180$ K was evaluated between coppers at 8.89 Å connected by two parallel bridges. One has a total length of 11.15 Å and contains five diamagnetic atoms, Cu−Br$_{eq}$···H−C−C−N$_{eq}$−Cu, including a weak hydrogen bond. The other contains a stacking between the pyrazole and phenyl rings. In CuCl$_2$(1-phenyl-3,5dimethylpirazole)$_2$,[22] an exchange coupling $|J|/k_B = 9(2) \times 10^{-3}$ K was evaluated between coppers at 8.33 Å connected by a bridge of seven diamagnetic atoms, Cu−Cl$_{eq}$···H−C−C−C−N−N$_{eq}$−Cu, having total length of 13.60 Å, which includes a weak hydrogen bond. In the complex aqua(glycyl-L-tryptophanate)-copper(II) dihydrate,[14] exchange interactions between Cu ions with magnitudes 12×10^{-3} K and 3×10^{-3} K were evaluated. The stronger one (type a) acts between a Cu ion and Cu neighbors at 8.38 and 8.47 Å. These paths include seven diamagnetic atoms with two moderate H bonds through a nonbonded water molecule, Cu$_A$−O$_{eq}$···H···O$_w$···H−O−C−N$_{eq}$−Cu$_B$. The distances along the bonds are 11.90 and 11.77 Å, and the two paths differ in the type of water molecule involved in the H bond. The weaker interaction is sustained by a bridge (type b) connecting Cu ions at 8.78 Å, containing 10 diamagnetic atoms, Cu−O$_{eq}$−C−O···H−N−C−C−C−C−N$_{eq}$−Cu, including a moderate H bond with a total length of 16.69 Å.[14] Recently, we reported[15] an exchange coupling $|J|/k_B = 9 \times 10^{-3}$ K between Cu ions at 14.09 Å connected by a long bridge of 12 atoms (distance along the path $d' = 19.18$ Å), Cu−N$_{eq}$−C−C−C−C−N−C−N−H···O$_w$···H−N$_{eq}$−Cu, including two moderate hydrogen bonds in the complex [Cu(II)(L-arginine)$_2$(SO$_4$)·(H$_2$O)$_6$]. This information and the value obtained here for Cu(II)Ala-Phe are included in Table 2, where the compounds

---

(19) A ferromagnetic interaction with a magnitude $J/k_B \sim 3$ K between copper ions connected by a similar bridge was evaluated in a Cu−peptide compound[13] using magnetic measurements.

(20) The great advantage of the method used here to evaluate an extremely small exchange coupling is that the exchange narrowing phenomenon (ref 4) operates on (and averages out) dipolar and hyperfine interactions, but it does not over exchange couplings. This allows selective measurement of small exchange couplings in the presence of much larger couplings (in our case, a 3 orders of magnitude difference).

(21) Sartoris, R. P.; Ortigoza, L.; Casado, N. M. C.; Calvo, R.; Castellano, E. E.; Piro, O. E. *Inorg. Chem.* **1999**, *38*, 3598−3604.

(22) Costa-Filho, A, J.; Munte, C. E.; Barberato, C.; Castellano, E. E.; Mattioli, M. P. D.; Calvo, R.; Nascimento, O. R. *Inorg. Chem.* **1999**, *38*, 4413−4421.

are ordered by the number of diamagnetic atoms in the bridge. We classify these compounds in three groups. Group I includes cases **1** and **2** with five diamagnetic atoms. Group II includes cases **3** and **4a** with seven diamagnetic atoms, and group III includes cases **4b**, **5**, and **6** with 10 or more atoms. The interactions corresponding to bridges in group I are 5−10 times larger than those for bridges in group II, even if the distances d between copper ions and the lengths d' along the bridges are similar. This may indicate that, for few atoms in the bridge, $|J|$ strongly depends on their structural details (i.e., on the several intermediate sections of the bridge) and not only on their lengths. The distances d and d' are similar within group II and so are the exchange couplings $|J|$. Within group III, the values of d' are much longer than those for the cases in group II, and the values of $|J|$ are smaller, except for in case **6**. In that case (see Table 2), the bridge is similar to that connecting the two quinone acceptors, Q_A^- and Q_B^-, at a distance of 17.3 Å in the bacterial photosynthetic reaction center protein of *Rhodobacter sphaeroides*, where an exchange interaction of 3.8 mK has been evaluated.[9,23] This indicates that, in principle, one could rely on simple model systems such as Cu(II)Ala-Phe and others to predict magneto-structural correlations valid for biological macromolecules. Because the magnitudes of the exchange couplings are related to the matrix elements for electron transfer,[7−9,11] this approximation offers a valuable procedure to estimate values of the electron-transfer matrix elements, which can be extrapolated to proteins.

A distance dependence of the magnitude of the exchange couplings was first proposed by Coffman and Buettner[24] and later updated by Hoffmann et al.,[25] who included new experimental information. More recently, a distance dependence of the magnitude of the exchange interaction supported by several long chemical bridges connecting cofactors that become paramagnetic during the photosynthetic process in the reaction center of *R. sphaeroides* (an electron-transfer protein) has been reported.[26] When the bridges are long, the results for the Cu amino acid and Cu peptide model systems included in Table 2 also support an exponential distance dependence of the exchange coupling. A second step in the process of modeling the magnitude of the exchange interaction J provided by a bridge containing p segments (σ bonds or contacts) would be the function

$$J = J_0 \exp(-\sum_{i=1}^{p} \eta_i) \tag{6}$$

where J_0 could be estimated from experimental information and η_i is a positive number which is small for strong covalent bonds (thus producing a small reduction of J) and large for H bonds or other weak bonds in the path. When the bridging system supporting the exchange interaction includes many atoms (and many bonds), eq 6 simplifies and gives a distance dependence that averages the individual characteristics of each bond. In a general case, eq 6 would give a better description of the change of J with the detailed properties of the bond and may have a broader applicability when more experimental results become available.

Acknowledgment. We are very grateful to Prof. O. R. Nascimento for his help with this work. This work is financially supported by a joint project PRONEX/FAPESP/CNPq (Grant No. 03/09859-2) in Brazil and by ANPCyT 06-13872, CAI+D-UNL, and CONICET PIP 2259 and 5274 in Argentina. A.J.C.F. thanks CNPq for a research fellowship. R.C. is a member of CONICET. The authors acknowledge the Spanish Research Council (CSIC) for providing a free of charge license to the CSD system.[27,28]

IC051957B

---

(23) Calvo, R.; Isaacson, R. A.; Paddock, M. L.; Abresch, E. C.; Okamura, M. Y.; Maniero, A. L.; Brunel, L. C.; Feher, G. *J. Phys. Chem. B* **2001**, *105*, 4053−4057.

(24) Coffman, R. E.; Buettner, G. R. *J. Phys. Chem.* **1979**, *83*, 2387−2392.

(25) Hoffmann, S. K.; Hilczer, W.; Gozlar, J. *Appl. Magn. Reson.* **1994**, *7*, 289−321.

(26) Calvo, R.; Isaacson, R. A.; Abresch, E. C.; Okamura, M. Y.; Feher, G. *Biophys. J.* **2002**, *83*, 2440−2456.

(27) Allen, F. H. *Acta Crystallogr., Sect. B* **2002**, *58*, 380−388.

(28) Bruno, I. J.; Cole, J. C.; Edington, P. R.; Kessler, M.; Macrae, C. F.; McCabe, P.; Pearson, J.; Taylor, R. *Acta Crystallogr., Sect. B* **2002**, *58*, 389−397.