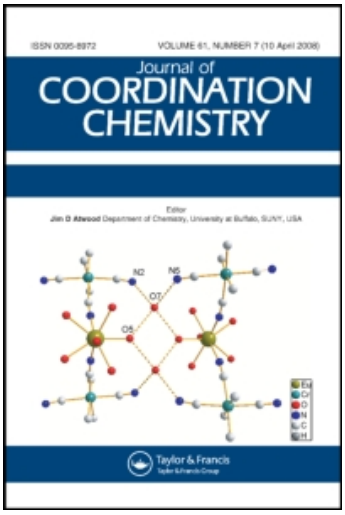

Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

Spectroscopic Studies of Metal Ion Mediated Interactions with Components of Nucleic Acids. I. Esr Study of Ternary Copper(II) Complexes ofα-Amino Acids and Dipeptides with Adenosine

Milan Strašák[a]; Zlatica Durcová[a]
[a] Department of Analytical Chemistry, Faculty of Pharmacy, Comenius University, Bratislava, Czechoslovakia

To cite this Article Strašák, Milan and Durcová, Zlatica(1990) 'Spectroscopic Studies of Metal Ion Mediated Interactions with Components of Nucleic Acids. I. Esr Study of Ternary Copper(II) Complexes ofα-Amino Acids and Dipeptides with Adenosine', Journal of Coordination Chemistry, 22: 2, 99 — 107
To link to this Article: DOI: 10.1080/00958979009410032
URL: http://dx.doi.org/10.1080/00958979009410032

J. Coord. Chem. 1990, Vol. 22, pp. 99–107
Reprints available directly from the publisher
Photocopying permitted by license only

SPECTROSCOPIC STUDIES OF METAL ION MEDIATED INTERACTIONS WITH COMPONENTS OF NUCLEIC ACIDS. I. ESR STUDY OF TERNARY COPPER(II) COMPLEXES OF α-AMINO ACIDS AND DIPEPTIDES WITH ADENOSINE

MILAN STRAŠÁK* and ZLATICA DURCOVÁ

Department of Analytical Chemistry, Faculty of Pharmacy, Comenius University, 832 32 Bratislava, Czechoslovakia

(Received February 5, 1990)

The formation of the title ternary complexes in aqueous solution has been investigated by electronic and ESR spectroscopy. ESR parameters have been used to compute molecular orbital coefficients. Coordination modes and their influence on metal-ligand bonding have been studied. A characteristic difference between GlyPro and other dipeptides is attributed to the lack of the peptide proton in GlyPro. It is suggested that, at near physiological pH conditions, the nucleoside binds at an equatorial site by displacing a water molecule from the Cu(II) ion.

Keywords: Copper(II), ESR, amino acid, dipeptide, adenosine

INTRODUCTION

The selective recognition of nucleic acids by proteins requires direct interactions between the chemical groups constituting each of the two macromolecules. However, indirect interactions mediated either through space or through metal ions could also participate in the formation of protein–nucleic acid complexes. For example, zinc(II) ions are known to be inherent components of many enzymes involved in nucleic acid metabolism.[1] In RNA and DNA, the phosphate group is only weakly basic and is a much weaker metal binding site than in nucleotides. Therefore, nucleosides provide suitable models for specific metal ion binding to extended nucleic acid polymers, when the negative charge at the phosphate is compensated by a background cation.[2]

In order to provide experimental evidence for this type of interaction, we have investigated the formation in solution of ternary complexes mediated through Cu(II) ions and involving adenosine on the one hand, and dipeptides or α-amino acids on the other. The crystal structures of some binary copper(II) complexes with peptides[3] or nucleosides[4,5] are known. ESR techniques can be employed to detect the nature of the primary coordination sphere in Cu(II) complexes since the Spin Hamiltonian parameters obtained can be used to establish symmetry and bonding properties of the complexes. As few ESR studies of Cu(II) ternary complexes with nucleosides or nucleotides with dipeptides have appeared in the literature,[6–8] and the bonding sites for histidine,[9–12], tryptophan,[12] and adenosine[2] are not unambiguous, we undertook an investigation of the ESR and electronic spectra of these complexes in order to obtain structural information on metal-ligand bonding.

* Address for correspondence.

EXPERIMENTAL

L-Amino acids of analytical grade from Fluka were used without further purification. Dipeptides glycylglycine (GlyGly), glycyl-*L*-leucine (GlyLeu) and glycyl-*L*-tryptophan (GlyTry) were of Reanal and 99.7% chromatographic grade; glycyl-*L*-proline (GlyPro) of analytical grade was kindly supplied free of charge by Serva. Histamine (Hm) was supplied by Merck. Adenosine (A) was purchased from Loba Chemie. All other chemicals were of the highest grade available. The spectra of the ternary species were obtained in aqueous solutions containing copper(II), adenosine, and the amino acid or dipeptide, respectively, in the molar ratio 1:1:1 (concentration: 5×10^{-3} mol dm^{-3}) at pH 6.5–7.5. No buffers were used, in order to avoid interaction of Cu(II) with other ligands. Solutions for optical absorption measurements were prepared by slowly adding concentrated NaOH (2 M) to aqueous solutions initially at pH 3. Under the conditions employed, the change in volume of the solutions was negligibly small. A Radiometer PHM-4 pH meter with glass and SCF electrodes was used for the above adjustments.

ESR spectra were recorded on an ERS 230 (Academy of Science GDR, ZWG Berlin) instrument. First derivative ESR X-band (*ca* 9.4 GHz) spectra were obtained; microwave power 5 mW, modulation amplitude 0.5 mT. Both solution and glass spectra were obtained using DMSO–water mixtures (1:5 and 1:1 v/v, respectively) at 295 or 103 K, respectively, to avoid aggregation of the Cu(II) complexes. The g values were calibrated against polycrystalline α,α′-diphenyl-β-picrylhydrazyl radical (DPPH). For solution spectra measurements, flat cells were used. Electronic spectra were measured with a Specord M 40 spectrophotometer (Carl Zeiss), glass cells being used. All solutions were incubated at 35°C for 2 h, then cooled to 25°C and the spectra recorded.

ESR results were evaluated by assuming an effective D_{4h} local symmetry for the complexes. In this case, the symmetry-adapted antibonding molecular orbitals of the Cu(II) ion can be written[13] as shown in (*1*) to (*4*),

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

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

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

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

where α, β_1 and β are the coefficients which express the covalent character of the in-plane σ-bonding, in-plane and out-of-plane π-bonding, respectively, and the other symbols have their usual significance. In the case of elongated octahedral structures, the unpaired electron is placed in the $\psi^*_{B_{1g}}$ orbital. These coefficients can be obtained from the relations which connect them with the Spin Hamiltonian parameters for axial symmetry[13,14] by the use of an iterative procedure,[13]

$$g_\parallel = g_e - 8\lambda_o(\alpha^2\beta_1^2 - 0.04)/\Delta E_{xy} \tag{5}$$

$$g_\perp = g_e - 2\lambda_o(\alpha^2\beta^2 - 0.04)/\Delta E_{xz,yz} \tag{6}$$

$$A_{\parallel} = P[-K - 4\alpha^2/7 + (g_{\parallel} - g_e) + 3(g_{\perp} - g_e)/7] \quad (7)$$

where $g_e = 2.0023$ is the free electron g-factor, λ_o is the spin-orbit coupling constant ($-828\,cm^{-1}$), and P is the dipole coefficient ($0.036\,cm^{-1}$) of the free Cu(II) ion. ΔE_{xy} and $\Delta E_{xy,yz}$ are the electronic transition energies $^2B_{2g} \leftarrow {}^2B_{1g}$ and $^2E_g \leftarrow {}^2B_{1g}$, respectively. K is the Fermi hyperfine contact term, calculated from (*8*).

$$K = -A_o + P(g_o - g_e) \quad (8)$$

The parallel and perpendicular components of the g and A tensors obtained from the glass spectrum were used in equations (*5*) to (*7*), while ΔE was determined from the visible spectra.

The σ-bonding orbital between the ligand and the metal 4s orbitals can be written[15] as shown in (*9*).

$$\psi_{a_{1g}} = \varepsilon' 4s + \varepsilon\varphi_L(a_{1g}) \quad (9)$$

The MO coefficient ε′ which characterizes the covalent character of the 4s σ-bond in the case of effective D_{4h} symmetry, can be calculated from the Fermi hyperfine contact term according to (*10*),

$$K = \alpha^2(K_{core} - \varepsilon'^2 K_{4s}) \quad (10)$$

where K_{core} ($0.0185\,cm^{-1}$) and K_{4s} ($-0.015\,cm^{-1}$) are the core and outer s shell spin polarizations, respectively,[15] while α^2 is obtained from the (*7*). The value of α'^2, the out-of-plane σ-bond strength, was calculated using the relationship (*11*),

$$\alpha^2 + \alpha'^2 - 2\alpha\alpha' S = 1 \quad (11)$$

where S is the overlap integral which is taken as 0.093.[13]

RESULTS AND DISCUSSION

Visible Absorption Spectra

The coordination sphere of copper(II) in binary Cu(II)–amino acid and Cu(II)–dipeptide complexes is relatively well known.[3,12] The equatorial plane of the terdentate dipeptide complex contains, at a pH near 7, peptide nitrogen, amine nitrogen and carboxylic oxygen atoms; the fourth position is occupied by a water molecule. A second solvent molecule is often coordinated in an axial position. Results of a spectral study[12] indicate that histidine and tryptophan coordinate equatorially through amino- and heterocyclic nitrogen atoms in square-planar sites with the carboxylate oxygen atom in an apical position; the two remaining equatorial positions are occupied by water molecules.

In the visible spectra of tetragonal Cu(II) complexes, all three d–d transitions are closely spaced and occur under one absorption band (Table I). As the investigated complexes are assumed to have approximate D_{4h} symmetry, the splitting of the ^2E_g level is not expected to be large and the following possible energy level sequence may arise:[16] $^2B_{1g} < {}^2A_{1g} < {}^2B_{2g} < {}^2E_g$. The transition $^2E_g \leftarrow {}^2B_{1g}$ is likely to be the most

intense transition. The uncertainty involved in the assignment of $\Delta E_{xz,yz}$ (and $g_\perp$) usually precludes a reliable estimate of the out-of-plane π-bonding parameter β^2. However, even in cases where $\Delta E_{xz,yz}$ is not known accurately, a 20% error in $\Delta E_{xz,yz}$ values causes only about a 5% error in β.[13]

Upon addition of adenosine, a concomitant shift (~20 nm) of absorption to shorter wavelengths is observed. This change can be interpreted as being due to the displacement of a solvent ligand from the coordination sphere by the nucleoside nitrogen atom. This causes a shift of the absorption band to the higher energies as the ligand field increases.

TABLE I
ESR and ligand field spectral data for the ternary complexes.[a]

Complex	$g_\parallel$	$g_\perp$	$A_\parallel$	g_o	A_o	g_{av}[b]	$\lambda_{max}(\varepsilon_{max})$[c]	$^nN_{eq}$
[Cu(Gly)A]$^+$	2.246	2.073	191	2.147	55	2.132	660 (46)	2
[Cu(Pro)A]$^+$	2.282	2.073	168	2.149	65	2.145	646 (53)	2
[Cu(Phe)A]$^+$	2.264	2.061	180	2.160	58	2.131	663 (sh)	2
[Cu(Try)A]$^+$	2.265	2.048	176	2.122	75	2.123	620 (sh)	3
[Cu(His)A]$^+$	2.286	2.062	169	2.152	63	2.139	641 (45)	3
[Cu(Hm)A]$^+$	2.286	2.070	170	2.150	66	2.144	646 (56)	3
[Cu(GlyGly)A]	2.257	2.044	178	2.124	71	2.117	649 (60)	3
[Cu(GlyPro)A]$^+$	2.292	2.073	153	2.165	50	2.148	710 (35)	2
[Cu(GlyLeu)A]	2.206	2.045	167	2.106	77	2.100	626 (60)	3
[Cu(GlyTry)A]	2.204	2.043	169	2.104	76	2.098	620 (74)	3

[a] A values in units of 10^{-4} cm^{-1}; [b] $g_{av}=[\frac{1}{3}(g^2 + 2g^2)]^{1/2}$; [c] λ in nm, ε in l mol^{-1} cm^{-1}.

ESR Spectra

At room temperature, the species present in aqueous solution give rise to isotropic ESR spectra consisting of four (2I + 1) absorption peaks due to the coupling of the electron spin dipole (S = 1/2) with the copper nuclear spin dipole (I = 3/2). At 103K, typical glassy spectra were obtained, all of which were characterized by three well-resolved hyperfine lines on $g_\parallel$ and the complex shape of the perpendicular region. An "overshoot" signal at g *ca* 2.00, as is often the case in Cu(II) complexes,[17] accounts for intermediate orientations relative to the external magnetic field. All spectra are characterized by both $g_\parallel$ and $g_\perp$ values higher than 2.040, corresponding to a ground state configuration with the unpaired electron in the $d_{x^2-y^2}$ orbital. The spin-Hamiltonian parameters listed in Table I, such as the $A_\parallel$ values, and the shape of the perpendicular region in the glassy spectra are typical of tetragonal Cu(II) complexes with axial perturbation and strong in-plane ligands.[9,12]

For some of the ternary complexes investigated a difference between g_o obtained at room-temperature work and low-temperature g_{av} factors is observed. Since sufficient DMSO was used, aggregation of solute could be neglected. For the investigated binary Cu(II)–amino acid[10] as well as Cu(II)–peptide[6] systems, binuclear species could be excluded under the experimental conditions of the investigation. Typical ESR spectra recorded at room temperature are shown in Figures 1 and 2. The spectrum in Figure 1, nominally from [Cu(L)A]$^+$ (L = Gly), shows a high-field component arising from the presence of [CuL_2]; the shape of the low-field peak is also affected by the presence of some Cu^{2+} (aq), which in solution at room

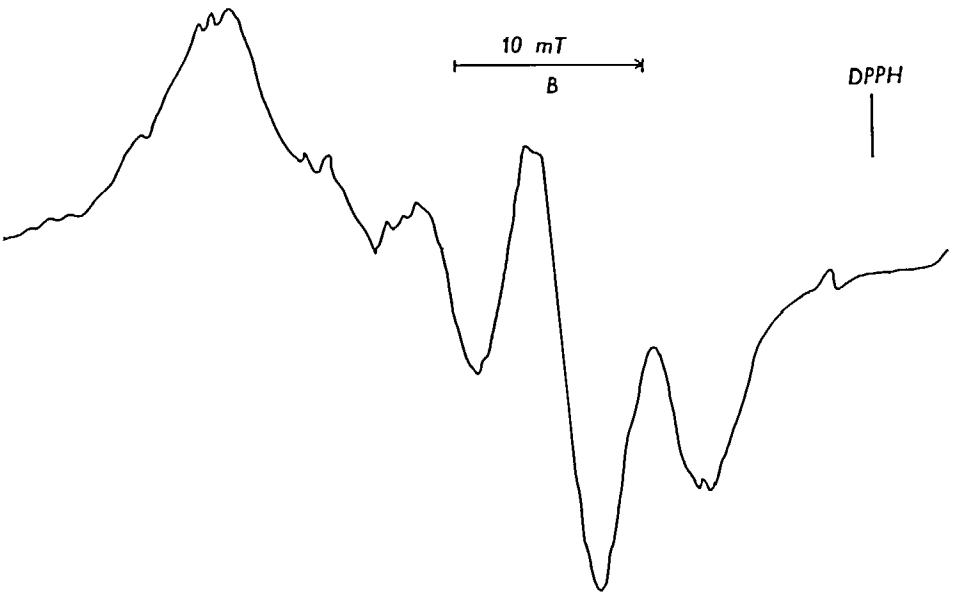

FIGURE 1 ESR spectrum of the ternary complex of adenosine with Cu(II)–Gly at room temperature; pH = 7.5. Other conditions as described in the experimental section.

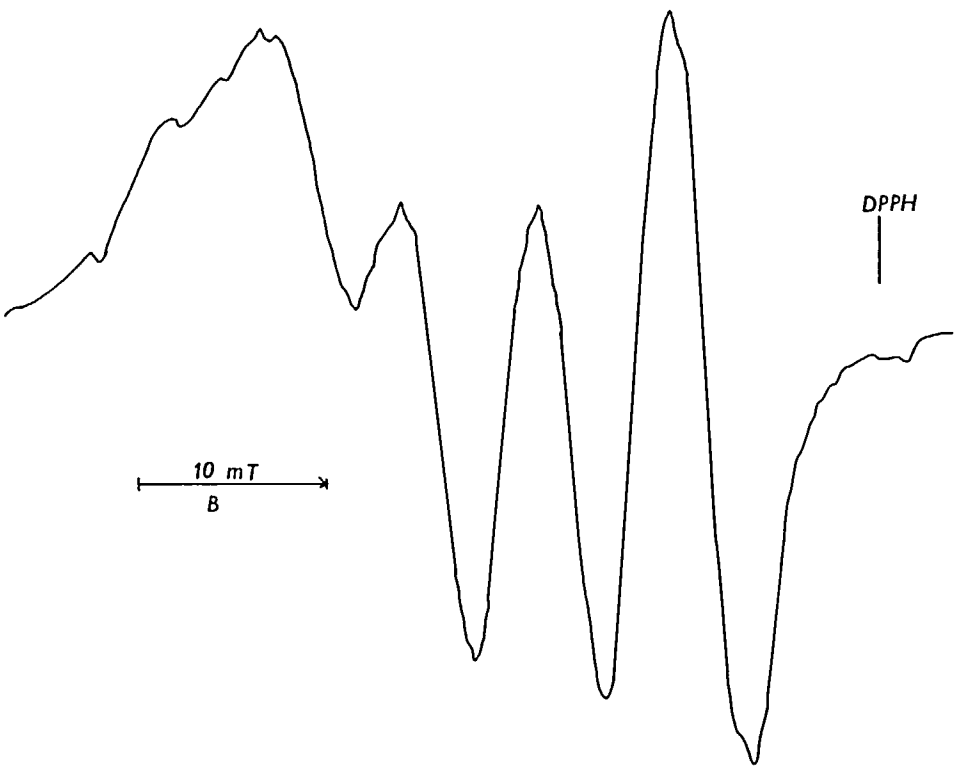

FIGURE 2 ESR spectrum of the ternary complex of adenosine with Cu(II)–GlyGly at room temperature. Other conditions are as described in the experimental section.

temperature gives a single broad peak. The spectra are essentially the same for the other ternary amino acid complexes, while for the dipeptide complexes (Fig. 2) only the low-field component is present. A similar situation was observed for binary Cu(II) complexes of parent amino acids and dipeptides.[12]

Typical glassy ESR spectra recorded at 103 K are shown in Figure 3. The use of various dipeptides does not lead to any significant changes in the form of the spectrum. The magnetic parameters of the ternary complexes derived from all the recorded spectra are given in Table I.

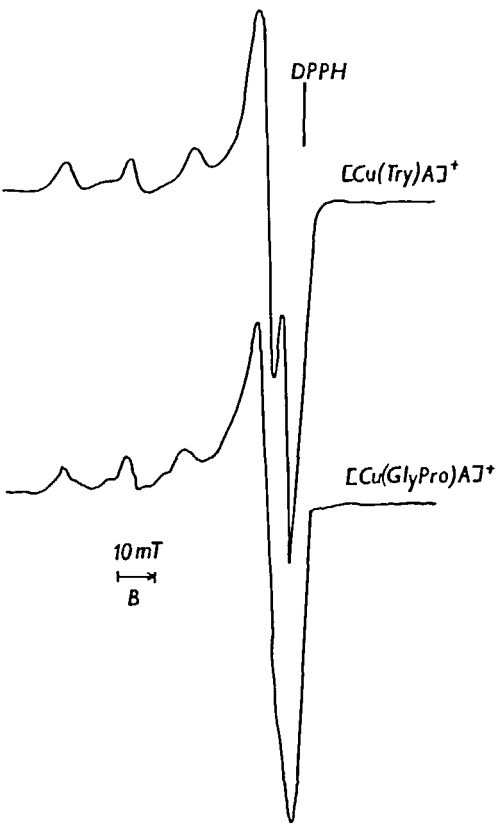

FIGURE 3 ESR spectra of ternary complexes in frozen solution; (a): $[Cu(Try)A]^+$; (b): $[Cu(Gly\text{-}Pro)A]^+$.

The difference between the frozen g_{av} and the water g_o factors may be due to the presence in the frozen samples of an additional bonded solvent molecule causing a distortion from tetragonal coordination. It seems that the magnitudes of both the anisotropic $A_{\parallel}$ and isotropic A_o values of the amino acid complexes are not directly proportional to the electron density on the copper. It would therefore appear that there is some involvement of the copper 4s orbitals in the molecular orbital that contains the unpaired electron, since the 4s electron density produces a contribution to the A values of opposite sign to that from the polarization of core s electrons by the electron density in 3d orbitals. The complex with the smaller A value has the greater unpaired electron density in the 4s orbital, if, as expected, core polarization

makes the major contribution to the A values. The different involvements of the 4s orbitals in the molecular orbitals containing the unpaired electron probably result from the different symmetries of the copper(II) ions in these complexes. The low symmetry in the complexes with the N_3O donor set would be expected to facilitate the mixing of the 4s orbital into the molecular orbital by a similar way as was observed in some Cu(II)–bis(amino acid) complexes.[18]

Further support for this conclusion comes from the calculated G values [given as the ratio $(g_{\parallel} - 2.002)/(g_{\perp} - 2.002)$] for the present complexes. According to the noted

variation of the G parameters (G = 3.44–6.07), the g values are markedly affected by exchange coupling. The G values calculated from the data for analogous ternary complexes of Cu(II) with peptides and nucleosides[7] are closely similar.

Almost all investigated complexes exhibit significantly smaller g parameters and significantly greater A parameters than the parent binary Cu(II)–amino acid and Cu(II)–dipeptide complexes, respectively.[12] This is consistent with an increase of covalent character due to coordination of adenosine. However, the ESR and ligand field parameters of [Cu(GlyPro)A] are markedly different from others of the dipeptide series. Proline contains no ionizable NH proton when it is inserted into a peptide chain. Hence the proline nitrogen atom in this case is a very poor donor. As a result, the proline residue in any position other than the *N*-terminal site may act as a "break-point" for metal coordination, thus allowing the two ends of the peptide chain to behave independently.[19]

TABLE II
Ligand field energies and molecular orbital coefficients for the complexes.

Complex	ΔE_{xy}, cm^{-1}	α^2	α'^2	β_1^2	β^2	ε'^2
$[Cu(Gly)A]^+$	15152	0.745	0.350	0.748	0.868	0.206
$[Cu(Pro)A]^+$	15480	0.716	0.389	0.913	0.923	0.096
$[Cu(Phe)A]^+$	15082	0.711	0.387	0.838	0.752	0.110
$[Cu(Try)A]^+$	16129	0.725	0.372	0.882	0.614	0.108
$[Cu(His)A]^+$	15600	0.744	0.351	0.896	0.819	0.138
$[Cu(Hm)A]^{2+}$	15480	0.744	0.351	0.891	0.851	0.124
[Cu(GlyGly)A]	15408	0.693	0.406	0.855	0.592	0.088
$[Cu(GlyPro)A]^+$	14085	0.705	0.393	0.874	0.853	0.143
[Cu(GlyLeu)A]	15974	0.726	0.371	0.678	0.571	0.135
[Cu(GlyTry)A]	16129	0.689	0.410	0.714	0.580	0.096

Metal-Ligand Bonds

The bonding parameters for the ternary Cu(II) complexes are given in Table II. Values of molecular orbital coefficients for these complexes show a fairly covalent character for each bond. As the coefficients α and β decrease, the bond becomes more covalent. Therefore the covalent nature of the copper–adenosine in-plane σ-bond of the ternary complexes decreases from dipeptides to amino acids. A linear relationship can be observed between ε'^2 and α^2 due to a competition between different types of σ-bonds, the first formed with the $3d_{x^2-y^2}$, the other with the 4s orbital.[15] This correlation has been found for several types of copper(II) complexes, *e.g.*, for amino acid complexes.[11,12] In our case (Fig. 4), deviation of some data is greater than experimental error. This can be explained by several factors. Overestimation of ε'^2

due to 3d–4s orbital mixing (see above), which occurs in the event of rhombic distortion, is possible. Thus, the fact that some of the ligands are bidentate while the other is terdentate in these complexes, seems to indicate a slight rhombic distortion of the ligand field in these species. Their MO coefficients should therefore be regarded as less accurate. This rhombic distortion is also reflected in the ternary complexes of some dipeptides (GlyLeu and GlyTry) by smaller A_{aniso} ($A_{\parallel}$–A_o) values (9.0 and 9.3 mT). The average A_{aniso} is 10.5 mT for the other complexes with N_3O donor atom sets. On the contrary, a rather more flattened tetrahedron is provided by the coordination sphere of Cu(II) in its ternary complex with GlyPro and adenosine as far as the parameter $f = g_{\parallel}/A_{\parallel}$[20] (150 cm) is observed. It was claimed[20] that f increases with increasing tetrahedral distortion (3d–4p orbital mixing). The f values for the other complexes (118–136 cm) are in the range expected for square-planar geometry (105–135 cm).[20]

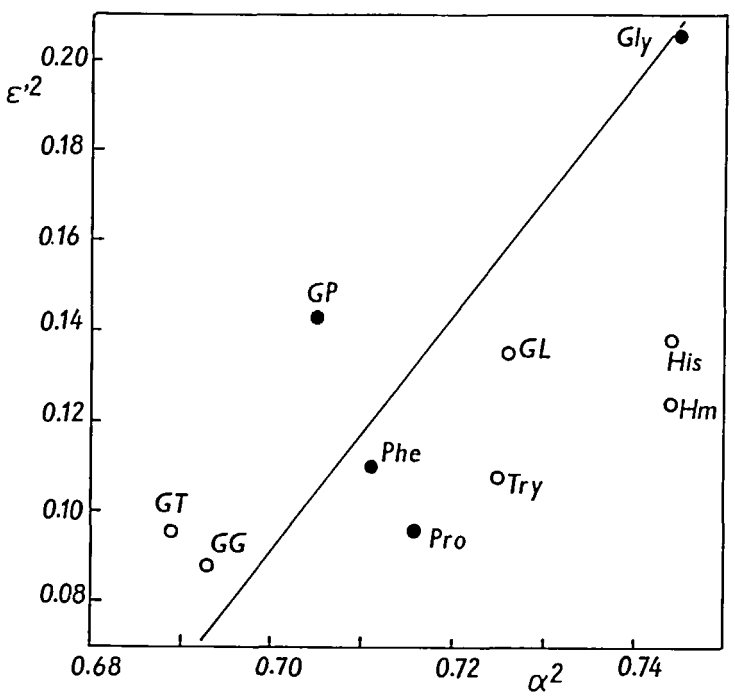

FIGURE 4 An ε'^2 *vs* α^2 plot for ternary copper(II) complexes of adenosine and amino acids or dipeptides, respectively. The solid line represents the best linear fit to data for complexes with effective D_{4h} symmetry; ●: Complexes with N_2O_2; ○: complexes with N_3O donor atom sets; GG, Glycylglycine; GL, glycyl-*L*-leucine; GP, glycyl-*L*-proline; GT, glycyl-*L*-tryptophane.

Purine nucleosides display a dichotomy between binding at N1 or N7.[2,21]. As a result, for adenosine, aqueous metal ion binding at both the N1 and N7 sites is important in the physiological pH range (6–8). This may be another source of error in the estimation of ε'^2.

Our spectral investigations indicate some rhombic distortion of the planar arrangement 3d–4s orbital mixing. In the ternary Cu(II)–peptide complexes as well as those of Cu(II)–amino acids with adenosine, the four equatorial positions are occupied by the terdentate peptide and N1 or N7 atoms of adenosine. The primary coordination sphere is completed by a solvent molecule in the axial position.

REFERENCES

1. A.S. Mildvan and L.A. Loeb, *CRC Crit. Rev. Biochem.*, **8**, 219 (1979).
2. R.B. Martin, In "Frontiers in Bioinorganic Chemistry", A.V. Xavier, Ed., (Verlag Chemie, Weinheim, 1986), p. 71.
3. H. Sigel and R.B. Martin, *Chem. Rev.*, **82**, 385 (1982).
4. H. Pezzano and F. Podo, *Chem. Rev.*, **80**, 365 (1980).
5. K. Maskos, *Wiad. Chem.*, **32**, 533 (1978).
6. F.R. Werner and B.M. Rode, *Inorg. Chim. Acta*, **91**, 217 (1984).
7. K. Maskos, *Acta Biochim. Polon.*, **32**, 13 (1985).
8. M. Strašák, in "Abstracts of the 6th CMEA Symposium on Electron Spin Resonance Spectroscopy in Biochemistry, Molecular Biology, and Medicine", Smolenice, 1988, p. 52.
9. P. Cocetta, S. Deiana, L. Erre, G. Micera and P. Piu, *J. Coord. Chem.*, **12**, 213 (1983).
10. B.A. Goodman, D.B. McPahil and H.K.J. Powell, *J. Chem. Soc., Dalton Trans.*, 822 (1981).
11. T. Szabó-Plánka, A. Rockenbauer, M. Gyor and F. Gaizer, *J. Coord. Chem.*, **17**, 69 (1988).
12. M. Strašák and Z. Durcová, *Collect. Czech. Chem. Commun.*, **55**, 546 (1990).
13. D. Kivelson and R. Neiman, *J. Chem. Phys.*, **35**, 149 (1961).
14. A.M. Maki and B.R. McGarvey, *J. Chem. Phys.*, **29**, 31, 35 (1958).
15. A. Rockenbauer, *J. Magn. Res.*, **35**, 429 (1979).
16. I.M. Procter, B.J. Hathaway and P. Nicholls, *J. Chem. Soc. A*, 1678 (1968).
17. T. Vanngard, in "Biological Application of Electron Spin Resonance," H. M. Swartz, J.R. Bolton and D.C. Borg, Eds., (Wiley-Interscience, New York, 1972), Chapter 9.
18. B.A. Goodman and D.B. McPhail, *J. Chem. Soc., Dalton Trans.*, 1717 (1985).
19. H. Kozlowski, G. Formicka-Kozlowska, L.D. Pettit and I. Steel, in "Frontiers in Bioinorganic Chemistry," A.V. Xavier, Ed., (Verlag Chemie, Weinheim, 1986), p. 638.
20. U. Sakaguchi and A.W. Addison, *J. Chem. Soc., Dalton Trans.*, 600 (1979).
21. R.B. Martin, *Acc. Chem. Res.*, **18**, 32 (1985).