Conformational Analysis of Bis(L-*N*,*N*-dimethylvalinato)copper(II) and Bis(L-*N*,*N*-dimethylleucinato)copper(II) in Different Solvents by EPR Spectroscopy and a Molecular Mechanics Study

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An EPR study on the Brownian motion of bis(L-*N*,*N*-dimethylvalinato)copper(II), Cu(L-$Me_2Val)_2$ and bis(L-*N*,*N*-dimethylleucinato)copper(II), Cu(L-$Me_2Leu)_2$, dissolved in different solvents (CD_3OD, CH_3CH_2OH, $CDCl_3$) as a function of temperature is presented. The theoretical fits of the EPR spectra revealed different EPR behaviour for the two copper(II) complexes in hydrogen bond making solvents. In order to examine whether the different behaviour could be explained on conformational (steric) grounds, a conformational analysis of the copper(II) complexes with L-*N*,*N*-dimethylated amino acids was performed with a molecular mechanics force field. The ability of the most stable conformations to bind a water molecule in the copper(II) first coordination sphere was also examined using the force field.

Introduction

Copper(II) complexes with amino acids and small peptides have been intensively studied in relation to their biological importance and possible pharmacological activity (here we refer only to few).[1−7] These compounds are also regarded as good model systems for copper binding sites in copper-containing enzymes and other proteins (e.g. prions).[8−10] Most of the biologically important interactions are achieved by the side-chains of the amino acids in proteins. These interactions may have a significant role in the oxidation state changes of the copper ion in copper-containing metalloproteins.[11]

Amino acid side-chain interactions such as those of the van der Waals type may also be important in the low molecular-weight copper(II) complexes with amino acids. Sigel and co-workers[12,13] studied amino acid side-chain interactions in the copper(II) complexes by glass electrode potentiometry. They pointed out that intramolecular van der Waals interactions formed by the two amino acid side-chains in the bis(chelated) copper(II) complexes could be formed only when 2N/2O donor amino acid atoms were arranged in a *trans* position in the equatorial plane of the copper(II) coordination sphere. Only the *trans*-CuN_2O_2 configuration was confirmed in a number of copper(II) complexes with L-*N*,*N*-dialkylated amino acids (other than glycine) dissolved in organic solvents by EPR spectroscopy[14,15] as well as by X-ray structural methods.[16−19] However, the *trans*-isomer of bis(glycinato)copper(II) was found to be more stable that the *cis*-isomer in the solid and gaseous phases, with a very quick isomerization reaction form *cis* to *tran*s in the gas phase at ambient temperature.[20]

The effect of water molecules dissolved in organic solvents on the electronic states of copper(II) complexes with L-*N*,*N*-dialkylated amino acids was studied.[15] It was suggested that if the amino acid side chains were long enough to form aliphatic intramolecular van der Waals contacts, then the presence of water in the organic solvent might induce the conformation change of the complex.

Thus, the effects of the water binding to the fifth (apical) position in the copper(II) coordination sphere on the length of the *N*-alkyl substituents in bis(chelates) with *N*,*N*-dialkyl amino acids were studied.[21,22] It was found that the water molecule bound to the copper(II) coordination sphere in the *N*,*N*-dialkylated alaninato complex could be easily released from the copper(II) but only when the *N*-alkyl chain was longer than methyl, whereas the apical water molecule was essential for the stability of the copper(II) complex with L-*N*,*N*-dimethylalanine.[21,22] However, EPR spectroscopic measurements of the copper(II) complexes of L-*N*,*N*-dimethylvaline or L-*N*,*N*-dimethylisoleucine revealed considerable influence of the water molecules dissolved in organic solvents on the resultant EPR spectra[15] and suggested that in these complexes, dissolved in organic solvents, the apically bound water molecule could be easily released from the copper(II) coordination sphere. Thus, considerable influence of the aliphatic amino acid residues on binding or release of

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the water molecule from the copper(II) coordination sphere could be envisaged.[22,23] Namely, a change in the conformation of the isoleucine side-chains in bis(L-*N*,*N*-dimethylisoleucinato)copper(II), i.e. Cu(L-Me_2Ile)$_2$, dissolved in deuterated methanol associated with the water molecule released from the copper(II) coordination sphere was suggested from the first study on the temperature dependence of the Brownian motion of the copper(II) amino acid complexes using EPR spectroscopy recorded at different frequencies.[22]

EPR spectroscopy is a powerful technique for the study of the electronic structures of the copper(II) complexes in order to gain information about the solute-solvent interactions as a function of the solvent, temperature and pH.[24,25] Interpretation of the results is sometimes difficult because of the lack of structural data. On the other hand, a recently proposed molecular mechanics (MM) force field (FFW) developed for modelling of anhydrous and hydrated copper(II) amino acid complexes[26] offers a unique possibility to gain qualitative insight into the arrangements that a chosen number of water molecules may adopt around the studied complexes. A combination of EPR spectroscopy and molecular mechanics calculations has become a valuable tool for identifying the preferred structure between different conformers.[27,28]

As a continuation of our previous investigations, we herein present an EPR study of the temperature dependence of the Brownian motion of bis(L-*N*,*N*-dimethylvalinato)copper(II), Cu(L-Me_2Val)$_2$, and bis(L-*N*,*N*-dimethylleucinato)copper(II), Cu(L-Me_2Leu)$_2$, dissolved in CD_3OD, CH_3CH_2OH and $CDCl_3$. These bis(L-dimethylamino acidato)copper(II) complexes have the two N-atoms in the copper(II) coordination sphere in the *trans*-position,[16−19] thus a similar temperature dependence of the ligand field parameters may be envisaged. The conformational analysis of these compounds was performed with the MM force field FFW[26,29] in order to find the most stable conformations. The ability of the most stable conformations to bind a water molecule in the copper(II) first coordination sphere (i.e. apical water) was examined. The combined EPR and MM studies are aimed at establishing whether different EPR behaviour of the copper(II) complexes examined in this work may be due to the aliphatic amino acid side-chain interactions, i.e. conformational (steric) in nature.

Results and Discussion

A) EPR Analysis

The EPR spectra and the rotational correlation time, τ_θ, of the complexes calculated from the spectra of Cu(L-Me_2Leu)$_2$ dissolved in CD_3OD and in $CDCl_3$, respectively, are compared in Figure 1. The spectra of the complex dissolved in one particular solvent could be fitted well with the same spectral parameters (Table 1) characteristic for that solvent in the whole temperature interval examined (i.e. 277−317 K in CD_3OD and 274−304 K in $CDCl_3$). The spectra of the complex dissolved in $CDCl_3$ (c and d) exhibit five well resolved superhyperfine lines due to the two nitrogen atoms in the copper(II) coordination sphere in the *trans*-position.[30,31] The resolved superhyperfine lines in the spectra also depend on the rotational correlation time, τ_θ.[24,32] A negligible influence of the anisotropic components ($a_{N\parallel}$ and $a_{N\perp}$) of the superhyperfine coupling tensor can be determined from spectrum c.[33,34] Well resolved superhyperfine lines suggest that the copper and nitrogen atoms are positioned in the equatorial plane of the copper(II) coordination sphere. The spectra of the complex dissolved in CD_3OD which are characterised by different EPR parameters (Table 1), albeit with the similar τ_θ values[24,32] as in $CDCl_3$, show five much broader superhyperfine lines in the whole temperature range studied. This is due to a displacement of the nitrogen atoms from of the copper(II) equatorial plane most probably by the apical water molecule in the complex.[15,35] A larger hyperfine splitting constant a_o, concomitant with decreased g_o values (Table 1) was determined from the spectra c and d (Figure 1) in $CDCl_3$. A comparison of this with the values determined from the spectra a and b in CD_3OD suggests more covalent character for the Cu−ligand bonds of the complex in $CDCl_3$.[35] This is in accordance with the finding that small deviations from the square-planar coordination shifts the hyperfine splitting constant to smaller values (see Table 1).[36]

Figure 2 shows the EPR spectra of Cu(L-Me_2Val)$_2$ dissolved in $CDCl_3$ (a and b), CD_3OD (c and d) and CH_3CH_2OH (e and f) recorded at two temperatures and their corresponding theoretical fits. In $CDCl_3$ the spectra were fitted well with the same parameters (Table 1) in the whole temperature range studied. As in the case of the leuc-

Table 1. EPR parameters of the copper(II) complexes

	g_0	$g_\parallel$	$g_\perp$	a_0/mT	$A_\parallel$/mT	$A_\perp$/mT
Cu(L-Me_2Leu)$_2$						
CD_3OD (277−317 K)	2.133 ± 0.001	2.244 ± 0.001	2.078 ± 0.001	7.8 ± 0.1	17.8 ± 0.1	2.8 ± 0.1
$CDCl_3$ (274−304 K)	2.115 ± 0.001	2.243 ± 0.001	2.051 ± 0.001	9.4 ± 0.1	17.8 ± 0.2	5.2 ± 0.2
Cu(L-Me_2Val)$_2$						
CD_3OD (276−296 K)	2.129 ± 0.001	2.245 ± 0.001	2.071 ± 0.001	8.2 ± 0.2	18.5 ± 0.1	3.1 ± 0.2
CD_3OD (296−316 K)	2.127 ± 0.001	2.245 ± 0.001	2.068 ± 0.001	8.4 ± 0.1	18.5 ± 0.1	3.4 ± 0.2
C_2H_5OH (284−308 K)	2.128 ± 0.001	2.244 ± 0.001	2.070 ± 0.001	8.4 ± 0.1	18.4 ± 0.1	3.4 ± 0.1
C_2H_5OH (308−328 K)	2.125 ± 0.001	2.244 ± 0.001	2.067 ± 0.001	8.6 ± 0.1	18.4 ± 0.1	3.7 ± 0.1
$CDCl_3$ (264−304 K)	2.116 ± 0.001	2.250 ± 0.001	2.050 ± 0.001	9.5 ± 0.1	17.9 ± 0.1	5.3 ± 0.1

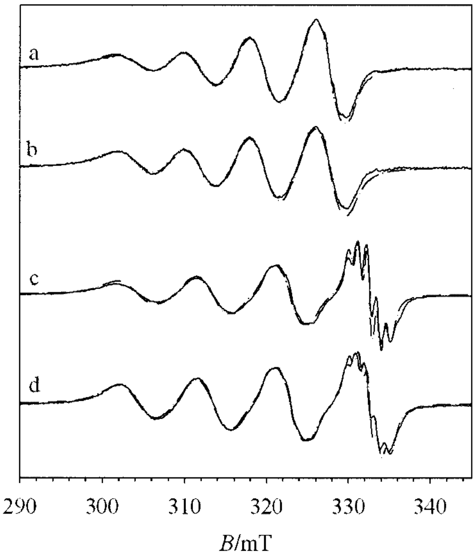

Figure 1. EPR spectra of Cu(L-Me_2Leu)$_2$ dissolved in CD_3OD taken at: (a) 294 K (τ_θ = 9.1·10^{-11} s), (b) 317 K (τ_θ = 7.0·10^{-11} s) and in $CDCl_3$ at (c) 274 K (τ_θ = 8.6·10^{-11} s), (d) 304 K (τ_θ = 8.0·10^{-11} s); full lines represent experimental spectra and dotted lines show the theoretical fit obtained with the EPR parameters a_o, g_o, $A_\parallel$ and $g_\parallel$ displayed in Table 1

inato complex, the spectra are characterised by well resolved superhyperfine structures due to the two nitrogen atoms in the copper(II) equatorial plane.

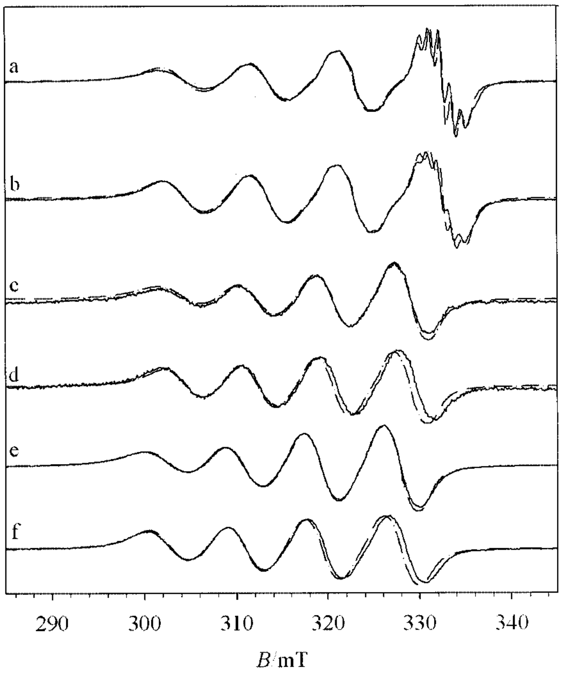

Figure 2. EPR spectra of Cu(L-Me_2Val)$_2$ dissolved in $CDCl_3$ at (a) 274 K (τ_θ = 6.6·10^{-11} s), (b) 304 K (τ_θ = 5.4·10^{-11} s); in CD_3OD at (c) 292 K (τ_θ = 6.9·10^{-11} s), (d) 316 K (τ_θ = 4.3·10^{-11} s); in CH_3CH_2OH at (e) 292 K (τ_θ = 7.1·10^{-11} s) and (f) 320 K (τ_θ = 4.0·10^{-11} s); full lines represent experimental spectra and dotted lines represent the theoretical fit with the EPR parameters of the lower-temperature ranges given in Table 1

However, in addition to the blurred superhyperfine structure of the spectra c−f (Figure 2), the theoretical fit of the spectra with the EPR parameters for the valine complex dissolved in CD_3OD and CH_3CH_2OH (i.e. in the hydrogen-bond making solvents) was satisfactory only in the lower temperature interval (see Table 1), assuming one type, the aqua complex, for the first copper(II) coordination sphere. In order to fit the experimental spectra d and f well (Figure 2), an increased a_o and a decreased g_o value, as determined from the experimental spectra were taken into account. This suggested an increase of the covalent character of the Cu−ligands bonds[36] of the complex represented by the experimental spectra d and f.

The reorientation correlation times, τ_θ, of the complexes which describe their Brownian motion in solution (see Methods) were calculated from the fitted spectra. Figure 3 shows a comparison of τ_θ calculated from the fit of the spectra of Cu(L-Me_2Leu)$_2$ and Cu(L-Me_2Val)$_2$ dissolved in CD_3OD.

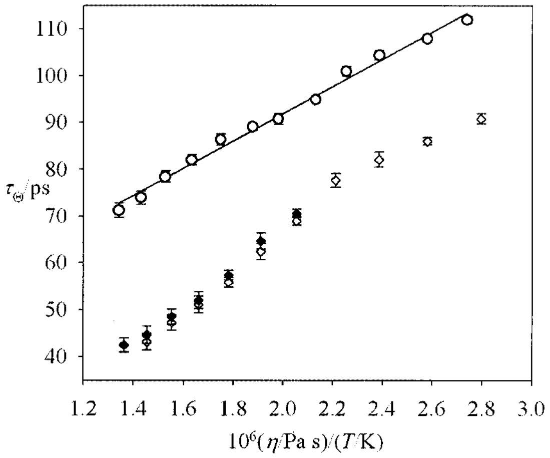

Figure 3. The reorientation correlation times, τ_θ, calculated from the fit of the spectra of Cu(L-Me_2Leu)$_2$ (white circles) and Cu(L-Me_2Val)$_2$ (white squares − fitted with the parameters required for the good fit of the spectra at the lower temperatures; black squares − fitted with parameters required for the good fit of the spectra at higher temperatures (see Table 1) dissolved in CD_3OD as a function of η/T

The linear dependence of τ_θ on η/T obtained with the parameters (Table 1) of the fitted spectra of Cu(L-Me_2Leu)$_2$ suggests an unchanged effective volume of the complex over the whole temperature range studied and indicates the presence of one conformation of the complex in the whole temperature interval examined. On the other hand, τ_θ calculated from the fit of the spectra of Cu(L-Me_2Val)$_2$ dissolved in CD_3OD, exhibited no linear dependence on η/T. An increase of a_o concomitant with a decrease of g_o which was determined from the spectra above 296 K (Table 1) is reflected in the function $\tau_\theta = f(\eta/T)$ as a deviation from linearity. Above 296 K increased a_o values (Table 1) suggest a higher covalent character of the Cu−ligand bonds whereas the nonlinearity of the function $\tau_\theta = f(\eta/T)$ suggests a changed solvated volume of the complex, indicating a conformation change.

When the two complexes were dissolved in $CDCl_3$ (Figure 4), a linear function of τ_θ against η/T was obtained and the spectra were fitted well with a single value for a_o and a single value for g_o (Table 1) in the whole range of temperatures examined. It can be seen that the values of τ_θ, calculated from the spectra of the complexes, as well as the slopes of the straight lines, $\tau_\theta = f[V_{eff}(\eta/T)]$, ($V_{eff}$ denotes the effective volume of the solvated complexes), are different. This result suggests that the motion of Cu(L-$Me_2Leu)_2$ is slower than that of Cu(L-$Me_2Val)_2$.

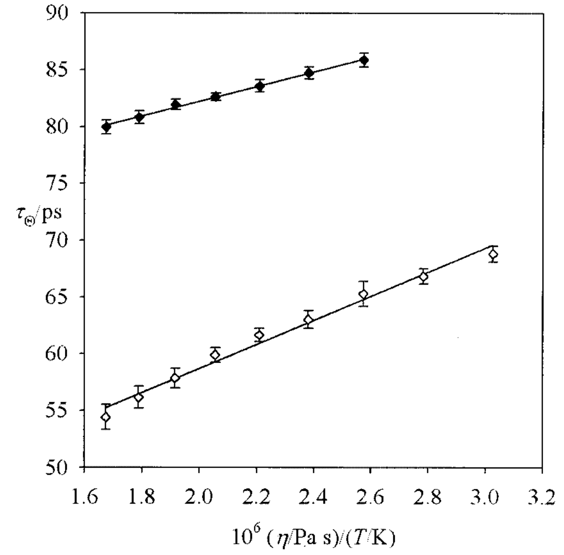

Figure 4. τ_θ calculated from the fit of the spectra of Cu(L-$Me_2Val)_2$ (white squares) and Cu(L-$Me_2Leu)_2$ (black squares) dissolved in $CDCl_3$ as a function of η/T using the parameters shown in Table 1

Cu(L-$Me_2Val)_2$ dissolved in CH_3CH_2OH exhibited a linear dependence of τ_θ on η/T (Figure 5), although an increase of a_o and a decrease of g_o were determined above 308 K. The linear dependence of τ_θ on η/T indicates that the solvated volume of the complex did not change above 308 K, although there was a change in the copper(II) electronic state resulting from an increase in the covalent character of the copper−ligand bonds.

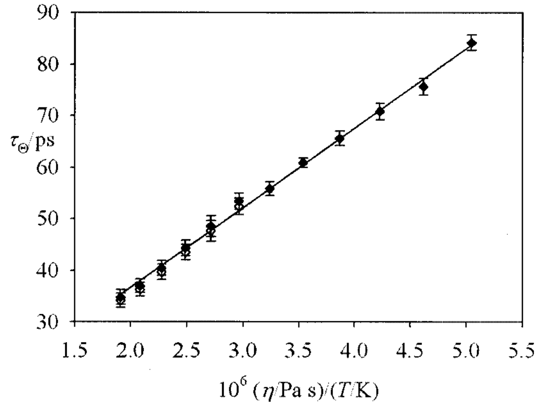

Figure 5. τ_θ calculated from the fit of the spectra of Cu(L-$Me_2Val)_2$ dissolved in CH_3CH_2OH as a function of η/T (black squares fit with $g_o = 2.128$, $a_o = 8.4$ mT; white squares fit with $g_o = 2.125$, $a_o = 8.6$ mT)

B) Conformational Analysis

The conformational analysis of Cu(L-$Me_2Val)_2$ and Cu(L-$Me_2Leu)_2$ were performed with the MM force field FFW[26,29] developed for anhydrous and hydrated copper(II) amino acid complexes.

From stereochemical considerations it can be proposed that each chelate ring of Cu(L-$Me_2Val)_2$ can have 6 conformations (3 conformers with the valine side-chains in the axial position and 3 conformers with them in the equatorial position with respect to the chelate ring planes) and that each chelate ring of Cu(L-$Me_2Leu)_2$ can have 18 conformations (9 conformers with the C^β-axial and 9 conformers with the C^β-equatorial positions). The results of in vacuo strain energy minimisations for the two systems, Cu(L-$Me_2Val)_2$ and Cu(L-$Me_2Leu)_2$, are listed in Table 2. Figure 6 shows their most stable conformations.

The conformational analysis was extended to the conformers interacting with water molecules (Table 2) in order to study the influence of amino acid side-chains on water binding in the first copper(II) coordination sphere. Due to the low number of water molecules dissolved in the studied organic solvents we limited the number of water molecules surrounding the copper complexes to 4. The initial atomic positions used for water oxygens and water hydrogens were the same for all the studied conformers.

The calculated equilibrium structures suggest that four water molecules may occur around the copper(II) amino acid complexes (Figures 7 and 8). It may be expected that FFW overestimates the distance between Cu and the axial water oxygen (denoted as O_w in Figures 7 and 8) by an average of 0.2 Å.[26] We suggest that every axially placed O_w at a distance longer than 3 Å cannot be considered as an apically bound water molecule.[37] The apically bound water molecule can be defined as the one that may influence the electronic structure of the copper(II), i.e. cause changes in the EPR spectral parameters.

The a6−a6 minimum of Cu(L-$Me_2Val)_2$ (Figure 6, Table 2) corresponds to the conformation of the anhydrous complex determined by the X-ray diffraction experiment.[16] The a6−e2 axial-equatorial minimum in the same compound (Figure 7) corresponds to the experimental crystalline conformation of the corresponding aqua-copper(II) complex.[18] The crystal structure of Cu(L-$Me_2Leu)_2$ has not yet been determined.

The conformers having the lowest strain conformational energies for the anhydrous complexes (Figure 6) also remained the most stable when four water molecules were present in the systems (Table 2). They have C^β in their axial positions as may be seen in Figures 6−8. Among the conformers with the same C^β-axial conformations in both chelate rings, only one conformer of Cu(L-$Me_2Val)_2$ (a5−a5, Table 2) can bind the apical water molecule in the first copper(II) coordination sphere when surrounded with more than two water molecules. In all other axial-axial conformers presented in Table 2, the methyl groups on the nitrogen atoms, as well as the close van der Waals contacts between two valine side-chains, prevent the apical water molecule

Table 2. Minimum strain energies for the conformers having two chelate rings in the same conformations for anhydrous complexes and for complexes surrounded with four water molecules

Conformer	φ_1 [a]	φ_2	φ_3	φ_4	Strain energy[b]	Strain energy
			Cu(L-$Me_2Val)_2$			Cu(L-$Me_2Val)_2\cdot 4H_2O$
				C^{β}-*equatorial*		
e1−e1	152.4	−143.7	167.6		14.92	20.55
e2−e2	147.4	−159.8	89.3		20.33	14.14
e3−e3	155.9	−153.1	−56.4		55.26	50.40
e1a−e1a	126.0	−152.7	176.1		22.39	29.34
				C^{β}-*axial*		
a4−a4	−158.6	−82.8	50.2		36.35	36.77
a5−a5	−154.7	−78.6	−91.2		10.03	7.53
a6−a6	−151.6	−89.7	−173.5		0.00	0.00
			Cu(L-$Me_2Leu)_2$			Cu(L-$Me_2Leu)_2\cdot 4H_2O$
				C^{β}-*equatorial*		
e1−e1	134.6	−144.4	−72.3	175.4	33.91	34.67
e1a−e1a	146.1	−165.5	−95.6	166.6	38.41	38.38
e2−e2	130.4	−139.2	−90.9	−35.3	92.27	92.19
e3−e3	155.5	−154.1	88.6	84.4	42.79	41.81
e4−e4	154.9	−150.0	−177.3	158.1	19.75	19.43
e5−e5	154.6	−149.2	−178.4	−74.9	32.27	34.62
e6−e6	138.3	−162.2	−174.2	68.5	11.61	15.14
				C^{β}-*axial*		
a7−a7	−155.2	−76.9	−94.2	−73.7	25.94	26.28
a8−a8	−146.6	−108.7	96.2	62.5	84.20	86.21
a9−a9	−151.6	−95.4	68.2	−157.9	26.25	29.22
a10−a10	−152.2	−79.8	−178.7	−77.3	6.91	12.91
a11−a11	−151.9	−80.2	177.8	49.8	16.05	15.88
a12−a12	−151.6	−80.1	172.6	−174.8	0.00	0.00

[a] Torsion angles $\varphi 1$, $\varphi 2$ and $\varphi 3$ correspond to O−Cu−N−C_{methyl}, $C^{\alpha}-C^{\beta}-N-$Cu and $C^{\gamma}-C^{\beta}-C^{\alpha}$-N (see Figure 6). Torsion angle $\varphi 4$ corresponds to the angle $C^{\delta}-C^{\gamma}-C^{\beta}-C^{\alpha}$ for Cu(L-$Me_2Leu)_2$ (see Figure 6). Torsion angles are in degrees. [b] Strain energies are in kcal mol^{-1}. Zero point energies: −4844.11 kcal mol^{-1} for Cu(L-$Me_2Val)_2$; −5337.15 kcal mol^{-1} for Cu(L-$Me_2Val)_2\cdot 4H_2O$; −5628.95 kcal mol^{-1} for Cu(L-$Me_2Leu)_2$ and −6124.77 kcal mol^{-1} for Cu(L-$Me_2Leu)_2\cdot 4H_2O$.

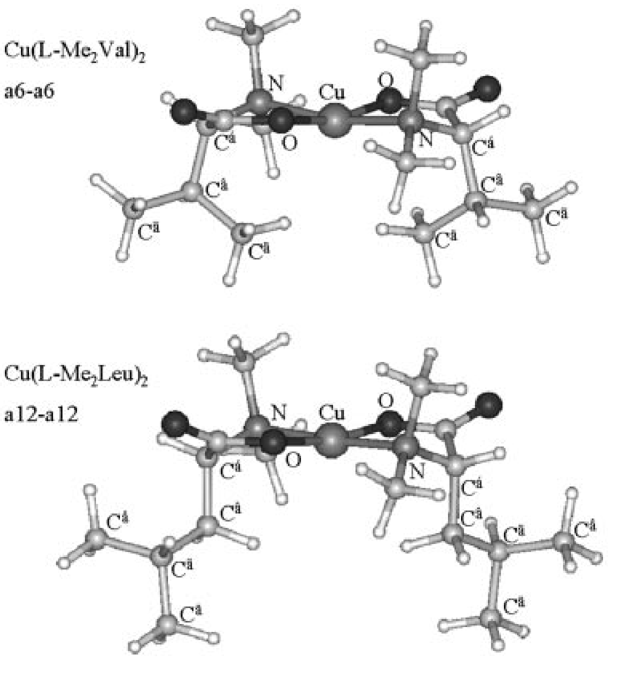

Figure 6. The most stable conformations of the two copper(II) complexes with L-*N,N*-dimethylated amino acids calculated with the FFW force field

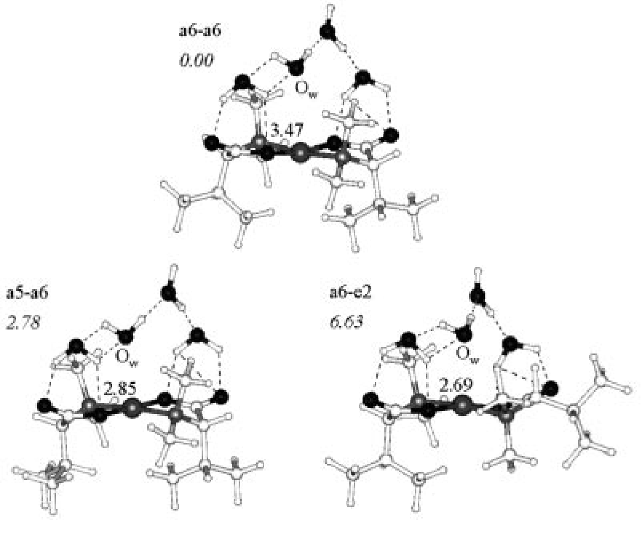

Figure 7. Three minima of Cu(L-$Me_2Val)_2\cdot 4H_2O$ with the lowest conformation energy; the values in italics are energy values expressed in kcal mol^{-1}; the values in Å are copper(II) to O_w distances

from approaching Cu^{II} (e.g. see a6−a6 in Figure 7). However, the conformers with C^{β}-equatorial in both chelate rings and in the mixed axial-equatorial positions do not hinder the water molecule from closely approaching the copper(II) (Figure 7).

On the other hand, almost all the minima of Cu(L-Me_2Leu)$_2$, including the most stable conformer a12-a12, can apically bind a water molecule to the axial position in the copper(II) coordination sphere when surrounded with more than two water molecules (Figure 8).

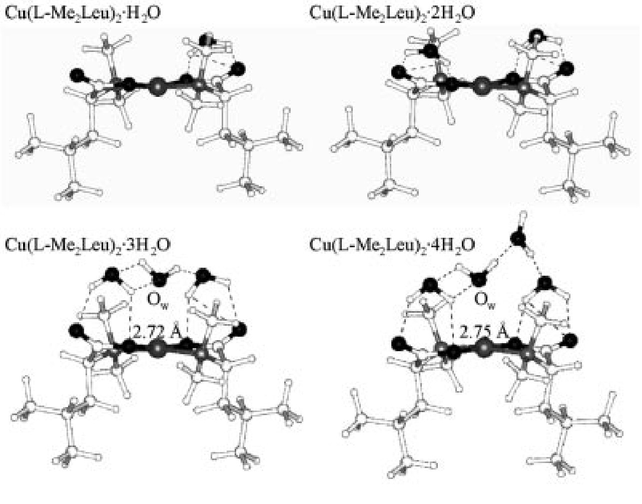

Figure 8. The a12−a12 conformer of Cu(L-Me_2Leu)$_2$ interacting with up to four water molecules; the values in Å are copper(II) to O_w distances.

According to the results obtained with the FFW force field, the interactions with four water molecules increase the energy differences between the most stable conformer and other conformations of Cu(L-Me_2Leu)$_2$, resulting in the a12−a12 as well as the11−a11 minima being even more stable than in the anhydrous system (Table 2). However, when four water molecules are present in Cu(L-Me_2Val)$_2$, the energy difference between the lowest energy minimum a6−a6 and the first above it becomes lower (Table 2 and Figure 7). These changes in the strain energy distributions among the conformers of the two theoretically examined systems, i.e. between the anhydrous system and the system containing four water molecules (Table 2), may suggest an even smaller energy difference between the hydrated conformers and the a6−a6 conformer of Cu(L-Me_2Val)$_2$ in more complicated system such as in real hydrogen-bond making solutions. With increased molecular vibrations at higher temperatures, the system may attain enough energy for the transformation from an equatorial-equatorial or an axial-equatorial to the axial-axial conformer, followed by the release of the water molecule from the copper(II) first coordination sphere. Such a conformer a12−a12 (the most stable for the two studied systems presented in Table 2, Figures 6 and 8) can easily accept and release the water molecule to and from the copper(II) coordination polyhedron.

Conclusions

The results of the EPR analysis of the temperature dependence of the Brownian motion point to different behaviour for Cu(L-Me_2Leu)$_2$ and Cu(L-Me_2Val)$_2$ dissolved in the hydrogen-bond making solvents CD_3OD and CH_3CH_2OH. This cannot be due to different temperature dependencies of the ligand field in the two complexes because both complexes have the same copper(II) coordination sphere.

For Cu(L-Me_2Leu)$_2$, a satisfactory match between the experimental and theoretical fits of the spectra with the EPR parameters characteristic for the chosen solution (Figure 1, Table 1) and the linear function $\tau_\theta = f(\eta/T)$ were obtained, suggesting an unchanged effective volume of the complex, i.e. an unchanged conformation of the side-chains.

For Cu(L-Me_2Val)$_2$, the unchanged effective volume was determined only from the spectra of the complex dissolved in $CDCl_3$. The non-linear dependence of τ_θ on (η/T) for Cu(L-Me_2Val)$_2$ in CD_3OD combined with changed a_o and g_o values above 296 K in CD_3OD, suggests a change in the effective volume of Cu(L-Me_2Val)$_2$ and consequently a different conformation. Different EPR parameters a_o and g_o observed for Cu(L-Me_2Val)$_2$ (Table 1) in the hydrogen-bond making solvents above a solvent dependent temperature reflect changes in the bonding and/or solvation of the complex. A decrease in g_o with a concomitant increase in a_o in the EPR spectra suggests more covalent character of the metal−ligand bonds in the equatorial plane of this complex. The isotropic hyperfine splitting constant a_o and the superhyperfine coupling constant a_N are highly sensitive to the geometric distortions. The changes in a_o and a_N determined from the spectra of Cu(L-Me_2Val)$_2$ on going from $CDCl_3$ to CH_3CH_2OH indicate pronounced flexibility of the copper(II) coordination polyhedron in this complex. This is in accordance with the findings[38] that with intramolecular and/or intermolecular strain the distortion of the copper(II) polyhedron from planarity was more energetically favourable than the changes in the valinato chelate rings' conformations. Thus, the non-linear dependence of τ_θ on (η/T) for Cu(L-Me_2Val)$_2$ in CD_3OD combined with a change in a_o suggests not only a change in the effective volume of Cu(L-Me_2Val)$_2$ above 296 K and consequently a different conformation, but also a differently distorted copper(II) coordination polyhedron.

The results of the theoretical conformational analysis offer an explanation for the differing EPR behaviour of Cu(L-Me_2Val)$_2$ and Cu(L-Me_2Leu)$_2$. The different behaviour may come from the lack of a bulky CH_3 group at C^β in Cu(L-Me_2Leu)$_2$ (Figure 6) compared with in Cu(L-Me_2Val)$_2$. The lack of the CH_3 group consequently allows the possibility of closer intramolecular contacts between aliphatic leucine side-chains in the two chelate rings upon water binding to the first copper(II) coordination sphere (Figure 8). That is, the most stable conformer of Cu(L-Me_2Val)$_2$ (a6−a6 in Figure 7) cannot accept a water molecule in the copper(II) first coordination sphere because of intramolecular steric effects. For Cu(L-Me_2Val)$_2$, we may conclude that the fifth copper(II) coordination site can be occupied only for some conformations and that the conformational changes (most probably triggered by solute-solvent interactions) can open and close the copper(II) accessibility to the water molecule. Conversely, Cu(L-Me_2Leu)$_2$ can bind and release the apical water molecule without the need for the conformational changes.

Experimental Section

Methods

A) EPR Spectroscopy: The EPR spectra of bis(amino acidato)copper(II) complexes are characterized by four resonances arising from the hyperfine coupling between an electron spin ($S = 1/2$) of the copper(II) (configuration $3d^9$) and the nuclear spin of the copper ($I_{Cu} = 3/2$), determined by the hyperfine coupling tensor $\hat{A}$ and the g-tensor related to the spin-orbit coupling. In solution, the average of the diagonal tensor values g_o and a_o is observed. Further splitting of the hyperfine lines may reveal the coupling of the copper electron spin with the nuclear spin of two nitrogens ($I_N = 1$) bonded to the copper(II)[30,31] resulting in the superhyperfine structures of the spectra (see Figure 1).

In this study we simulated the experimental EPR spectra of the complexes with the WIN-EPR Simfonia software (BRUKER) and the fast motion approximation. τ_θ was calculated from the linewidth (ΔB/mT) of the spectra which is dependent on the nuclear magnetic moment m_{Cu}:

$$\Delta B(m_{Cu}) = a + bm_{Cu} + cm_{Cu}^2$$

where a, b, c, are parameters for the lineshape fit.

The calculations of the reorientation correlation time, τ_θ, describing McConnell's tumbling mechanism[39] were performed following the procedure given elsewhere.[21]

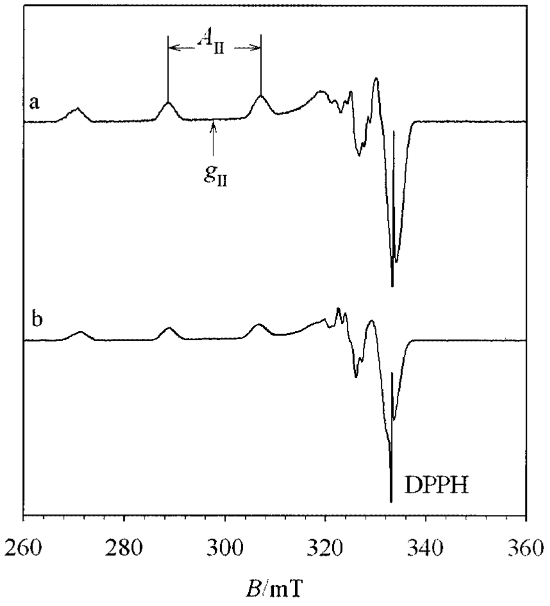

Figure 9. EPR spectra of (a) Cu(L-Me_2Val)$_2$ and (b) Cu(L-Me_2-Leu)$_2$, dissolved in CD_3OD and recorded at 105 K (see Table 1)

The reorientation correlation time, τ_θ, of the complex describes the Brownian motion in solution and may be expressed as $\tau_\theta = (1/k)\ V_{eff}\ (\eta/T)\ \kappa$, where V_{eff} is the effective volume of the complex, η/T the viscosity over temperature, k is the Boltzmann constant and κ is the anisotropic interaction parameter, the second rank tensor related to EPR spectral parameters.[40] If the complex retains one conformation in the examined temperature interval, τ_θ will be a linear function of η/T with the coefficient of linearity proportional to the effective volume of the dissolved complex.[22]

The parameters $A_\|$ and $g_\|$ required for the calculation of τ_θ were determined from the EPR spectra of the glassy state recorded at 105 K (Figure 9). The spectra indicate only one type of the copper(II) complex in solution with axial symmetry ($g_\| > g_\perp$) of the ligand field with the $d_{x^2-y^2}$ ground state.

EPR spectra were measured using a Varian E-109 spectrometer (X-band) equipped with a BRUKER variable temperature control unit. The g-factor was measured relative to diphenyl-picryl-hydrazil (DPPH) as an external marker ($g_{DPPH} = 2.0036 \pm 0.0003$). The temperature dependence of the viscosity η of the solvents was determined from published tables.[41]

B) Molecular Mechanics Calculations: The strain energy minimisations were performed with the CFF program for conformational analysis[42−44] with the FFW force field[26,29] suited for modelling anhydrous and hydrated copper(II) amino acid complexes with both a *trans*- and a *cis*-CuN_2O_2 coordination polyhedron. A detailed description of the potential energy functions used, the FFW's development as well as a discussion on the efficacy of the force field in simulation and prediction of experimental crystal and molecular structures and quantum-chemically calculated vacuum structures of a series of anhydrous and hydrated copper(II) amino acid complexes can be found in our earlier paper.[26] FFW correctly predicted the most stable conformations of the copper(II) amino acid complexes in vacuo and reproduced well the hydrogen-bonding pattern between water molecules and amino acid atoms.[26] It also simulates the result obtained from quantum chemical calculations for aqua-copper(II) glycinato systems[26,45] in which the formation of hydrogen bonds between water molecules and amino and carboxylato groups competes with axial $Cu\cdots OH_2$ interactions.

Materials:

The ligands L-*N*,*N*-dimethylvaline and L-*N*,*N*-dimethylleucine were prepared using a modified method of Bowman and Stroud[46] (i.e. by methylation of the amino acids through condensation with formaldehyde, followed by a catalytic hydrogenation in an ethanol/water solution at room temperature). The corresponding copper(II) complexes were prepared according to the modified procedure of Nash and Schaefer[47] (i.e. by means of the reaction of copper(II) acetate monohydrate with the sodium salt of the ligand in dichloroethane solution to obtain Cu(L-Me_2Val)$_2$, and in dry dichloromethane to obtain Cu(L-Me_2Leu)$_2$). The C, N and Cu elemental analyses confirmed the compositions of the ligands and complexes.

Concentrations of approximately 0.1 mmol dm^{-1} of the copper(II) complexes (in CH_2CH_3OH, $CDCl_3$, CD_3OD) were used. Organic solvents were dried over Type 4 Linde Na−Al-silicate molecular sieves with a pore radius of 4 Å (SERVA, Heidelberg, Germany).

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