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An Electron Paramagnetic Resonance Study of Copper(II)–β-Substituted β-Amino Acid Systems by the Two-Dimensional Simulation Method: First Evidence of Primarily Steric Effects of Substituents on Equilibria of Metal Complexes

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We have studied the complex equilibria of copper(II) with a series of β-substituted β-amino acids (R: H, Me, Et, *i*Bu, *i*Pr, *c*Hex, 1-EtPr, and *t*Bu) in aqueous solution by pH potentiometry and electron paramagnetic resonace (EPR) spectroscopy in the range pH = 2–8 at various metal and ligand concentrations. The basicities of the corresponding donor groups differed only slightly in the series of ligands. A purely mathematical method, the matrix rank analysis carried out on the EPR spectrum package recorded in the presence of copper(II), indicated the formation of 6 independent paramagnetic species. Accordingly, Cu^{2+} (aqua complex) and the complexes $[CuLH]^{2+}$, $[CuL]^{+}$, $[CuL_2H_2]^{2+}$, $[CuL_2H]^{+}$, and $[CuL_2]$ were considered in the subsequent analysis of series of spectra, and also two isomers of $[CuL_2]$ were identified. The formation constants and the EPR parameters, e.g. the isotropic *g*-factors and the copper and nitrogen hyperfine couplings for the above species, were determined in the same optimization procedure by the simultaneous evaluation of spectra. The ligands "LH" are suggested to bind in equatorial positions through their carboxylate groups, while the amino acids in the L protonation state are likely to occupy two equatorial sites via the amino and carboxylate groups. For the isomers of $[CuL_2]$, the donors of the same kind are in the cis or trans position. As far as we know, this is the first reported case in which a strong correlation has been found between the steric effects of substituents characterized by Meyer's steric parameter V^a and the protonation constants of metal complexes. The observed trend for the preference for nonprotonated complexes $[CuL]^{+}$ and $[CuL_2]$ to increase with the steric demand of the substituent was explained by the increasing shielding effect of the substituent hindering protonation of the nonprotonated complex.

Introduction

In the past two decades β-amino acids have emerged as a highly interesting class of compounds, primarily in consequence of their pharmacological properties.[1] They are present in humans, animals, plants, and microorganisms, either free or incorporated in peptides; some of the latter compounds have antibiotic, antifungal, or cytotoxic activities. β-Amino acids are fundamental moieties of the widely used β-lactam antibiotics and anticancer agents of taxane type, too. Their incorporation into peptides of pharmacological interest may exert a favorable effect on the biological activity and/or metabolic stability. These features can be modified by side-chain substituents.[2]

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The complexes of β-alanine with copper(II) and other transition metal ions of vital importance have been studied by several authors,[3] but the coordination ability of its β-substituted derivatives has not yet been examined, despite their pharmacological interest. Therefore, we have investigated the complex equilibria in solutions of copper(II) ion and various β-substituted β-amino acids, applying pH potentiometric and electron paramagnetic resonance (EPR) methods. We apply the EPR technique since for paramagnetic species it offers a more direct information on the coordination modes than any other spectroscopic method and, in particular, it is capable to distinguish the different microspecies (isomers). The large number of complexes in overlapping equilibria needs a special spectrum decomposition technique, the two-dimensional simulation developed recently,[4] which is based on the simultaneous analysis of series of EPR spectra recorded at various ligand-to-metal concentration ratios and pH's. Our simulation method treats the signal intensity simultaneously as a function of the magnetic field and the concentration distribution of the various species. This approach has two considerable advantages: (1) We can identify those complexes which are hardly discernible for pH potentiometry, since their formation is not accompanied by proton uptake or loss; moreover, apart from pH potentiometry or spectrophotometry, we can uniquely distinguish species with the same composition but different coordination. (2) We can provide not only the formation constants but also the EPR parameters, characteristic of the coordination modes, for each EPR-active species, including microspecies. Since the method can detect merely the paramagnetic metal complexes, the pH potentiometric formation constants of the proton complexes are necessary, and those of the metal complexes are useful as a good starting point in the iteration procedure of spectrum decomposition.

In this paper, we set out to clarify the microspeciation and coordination modes in 8 copper(II)−β-substituted β-amino acid systems and to study the effects of substituents of different sizes and structures on the complex equilibria.

Experimental Section

Materials. The (racemic) ligands were prepared as previously described.[5] Other reagents were of analytical grade from Reanal (Budapest, Hungary).

pH Potentiometric Measurements. The pH potentiometric titrations were performed in 0.2 M KCl at 25 ± 0.1 °C under a nitrogen flow with a Metrohm 765 Dosimat apparatus; pH was measured by a Radiometer PHN 240 potentiometer equipped with a Radiometer pHC2401-8 combined glass electrode. The electrode was calibrated with IUPAC standard buffers from Radiometer. Titrations in the absence of copper(II) ion were performed at 0.01 M initial ligand concentration (T_L) in the pH range 2.3−11.5. In the presence of copper(II) ion, T_L was 0.005 M, while the initial total copper(II) concentration (T_{Cu}) was 0.005, 0.0025, or 0.00125 M. Precipitation was observed in neutral or alkaline solutions, depending on the metal-to-ligand concentration ratio. The titration data were evaluated by the computer program PSEQUAD.[6]

EPR Measurements. The EPR spectra were recorded at 291 ± 0.5 K under an argon atmosphere at $T_{Cu} = 0.005$ M and $T_L = 0.025$ or 0.100 M, using an upgraded JEOL JES-FE3X spectrometer. We used 0.2 M KCl as background electrolyte. The pH was adjusted with HCl (0.2 M) and then NaOH (0.2 M) to an accuracy of 0.01 pH unit, using the same apparatus as for pH potentiometry. A Masterflex CL peristaltic pump ensured the circulation of the solution through the capillary tube in the cavity. The EPR spectra were recorded after 2 min of circulation at the chosen pH values, using a Mn(II)-doped MgO powder for the calibration of *g*. All spectra were recorded in 4000 data points with 24 bits precision. Further details of the measurement are given in a previous work.[7]

Precipitation was observed in all systems at pH ≥ 6, except for the copper(II)−β-alanine system where the measurement could be executed in the pH range 2.8−11.1. For the R = 1-EtPr and R = *c*Hex systems, EPR titrations had to be carried out at $T_{Cu} = 0.00125$ M and $T_L = 0.00625$ or 0.025 M, because of the low solubilities of the ligands and the complexes.

Evaluation of the EPR Spectra. The analysis of the spectra was preceded by the elimination of the background signal containing glass impurities and the peaks of the Mn(II) external standard and by a numerical field shift to obtain the spectra at a common frequency. The matrix rank analysis of spectrum packages was carried out by using the MRA program.[8] Then the series of EPR spectra were evaluated by using the 2D_EPR program.[4] The EPR spectra of the various species were described by the parameter g_0, the copper hyperfine coupling constant A_0, the N superhyperfine coupling constant a_{N_o}, and the relaxation parameters α, β, and γ relating to the line widths of the copper hyperfine multiplet as $W_{M_I} = \alpha + \beta M_I + \gamma M_I^2$ (M_I is the magnetic quantum number of copper nuclei). As we used a natural mixture of copper isotopes, the spectra were calculated as the sum of the curves of molecules containing isotope ^{63}Cu or ^{65}Cu weighted by their natural abundances. The hyperfine coupling constants and the relaxation parameters refer to the isotope ^{63}Cu. The coupling constants and the relaxation parameters are given in gauss (G) units throughout the paper; 1 G = 10^{-4} T.

The quality of the fit for the individual spectra was characterized by the noise-corrected regression parameter *R* computed from the average square deviation. The noise was deduced from the quadratic error of the fit to obtain $R = 1$ for a perfect fit. For the overall set of spectra, the overall regression parameter was applied, which is calculated from the sum of average square deviation values. The computer program also provides the critical difference in overall regression parameter, which can be regarded as significant between two speciation models. The details of the statistical analysis were given previously.[4]

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Results and Discussion

Speciation in the Copper(II)−β-Substituted β-Amino Acid Systems. For the great majority of the ligands shown in Figure 1, the pH potentiometric titration data could be described well in terms of the same simple speciation as assumed previously for the copper(II)−β-alanine system:[3] besides the proton complexes [LH] and $[LH_2]^+$, the species $[CuL]^+$ and $[CuL_2]$ were taken into consideration up to pH = 8. The corresponding formation constants are given in Table 1.

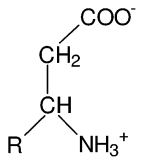

Figure 1. β-Amino acids studied: β-alanine (R = H); 3-aminobutanoic acid (R = methyl (Me)); 3-aminopentanoic acid (R = ethyl (Et)); 3-amino-5-methylhexanoic acid (R = isobutyl (*i*Bu)); 3-amino-4-methylpentanoic acid (R = isopropyl (*i*Pr)); 3-amino-3-cyclohexylpropanoic acid (R = cyclohexyl (*c*Hex)); 3-amino-4-ethylhexanoic acid (R = 1-ethylpropyl (1-EtPr)); 3-amino-4,4-dimethylpentanoic acid (R = *tert*-butyl (*t*Bu)).

The above pH potentiometric results suggest that the EPR spectrum packages (illustrated in Figure 2 for the ligand with R = *t*Bu) can be described with the assumption that 3 paramagnetic species (including Cu^{2+} as aqua complex) are formed in various systems. However, the results of matrix rank analysis (MRA), a model-free, purely mathematical treatment[8] of spectrum packages, indicate that more than 3 independent EPR-active complexes are present. In Figure 3, the residual intensities are illustrated which were obtained by MRA for the series of spectra for the ligand with R = *t*Bu (shown in Figure 2), assuming various numbers of independent paramagnetic species. At 3 independent complexes, the residual intensities at various fields exceed the noise considerably, and they decrease with increasing number of particles up to 6 independent species. This suggests the formation of 3 additional complexes.

Accordingly, in the two-dimensional EPR evaluation, we also had to include the protonated complexes $[CuLH]^{2+}$, $[CuL_2H_2]^{2+}$, and $[CuL_2H]^+$. Further, the spectra of $[CuL_2]$ had to be described as a superposition of two component curves; i.e., an isomeric equilibrium occurs for this complex. (For the other bis complexes, we could not detect isomeric equilibria.) Additionally, in the copper(II)−β-alanine system, the model was supplemented by the complex $[CuL_2H_{-1}]^-$ (which is formed at pH > 9.5). For the best models, good fits of the calculated and measured spectra were achieved: the noise corrected overall regression parameters[4] (*R*) lay between 0.99484 and 0.99779. The low standard errors

Table 1. Formation Constants as log β for the Copper(II) Complexes of the β-Substituted β-Amino Acids, Together with the Standard Errors of the Last Digits in Parentheses

	species								
method	[HL]	$[H_2L]^+$	$[CuLH]^{2+}$	$[CuL_2H_2]^{2+}$	$[CuL]^+$	$[CuL_2H]^+$	*cis*-$[CuL_2]$	*trans*-$[CuL_2]$	$[CuL_2H_{-1}]^-$
					R = H				
EPR			12.03(1)	22.83(3)	7.05(1)	18.90(2)	12.60(1) 12.77(1)[a]	12.28(1)	1.18(2)
pH pot.	10.11(1)	13.70(1)			7.00(1)		12.51(1)		
lit.[b]	10.14(5)	13.71(5)			6.99(7)		12.45(10)		
					R = Me				
EPR			11.81(1)	22.18(2)	7.10(1)	18.25(2)	12.46(1) 12.63(1)[a]	12.15(1)	
pH pot.	10.13(1)	13.55(1)			7.02(1)		12.80(2)		
					R = Et				
EPR			11.83(1)	22.30(1)	7.36(1)	18.56(1)	12.94(1) 13.11(1)[a]	12.63(1)	
pH pot.	10.14(1)	13.56(1)			7.17(1)		13.14(1)		
					R = *i*Bu				
EPR			11.48(1)	22.11(3)	7.11(1)	18.25(1)	12.74(1) 12.92(2)[a]	12.44(2)	
pH pot.	10.14(1)	13.57(1)			7.12(1)		13.06(2)		
					R = *i*Pr				
EPR			11.47(1)	21.87(2)	7.15(1)	18.10(1)	12.82(1) 12.94(1)[a]	12.32(1)	
pH pot.	10.03(1)	13.46(1)			7.12(1)		13.09(1)		
					R = *c*Hex				
EPR			11.42(1)	21.98(1)	7.23(1)	18.47(1)	12.96(3) 13.15(6)[a]	12.69(15)	
pH pot.	10.17(1)	13.65(1)			7.33(1)		13.38(5)		
					R = 1-EtPr				
EPR			11.55(1)	22.15(1)	7.20(1)	18.45(1)	12.98(3) 13.24(5)[a]	12.90(10)	
pH pot.	10.10(1)	13.59(1)			7.23(5)		13.25(8)		
					R = *t*Bu				
EPR			11.39(1)	21.99(1)	7.16(1)	18.36(1)	13.08(1) 13.33(1)[a]	12.97(1)	
pH pot.	9.95(1)	13.29(1)			7.33(1)		13.22(2)		

[a] Overall formation constants, $\log \beta = \log(\beta_{\text{cis isomer}} + \beta_{\text{trans isomer}})$. [b] Average of the pH potentiometric data from ref 3.

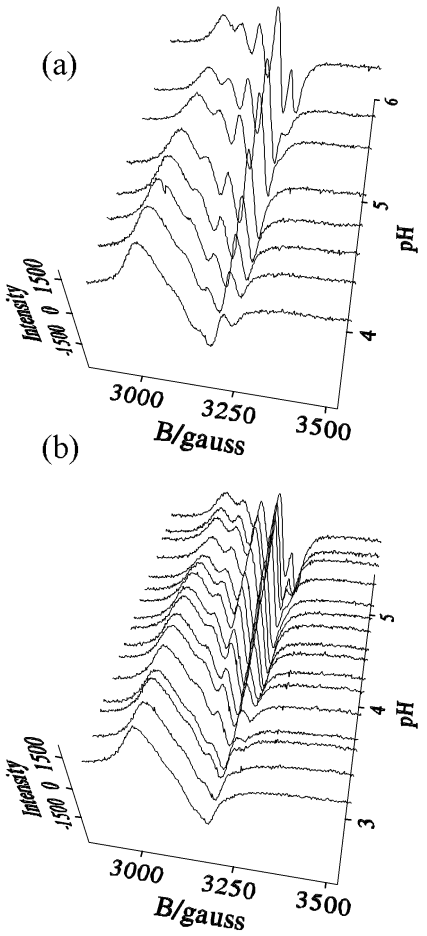

Figure 2. Series of experimental EPR spectra for the copper(II)−3-amino-4,4-dimethylpentanoic acid (R = *t*Bu) system: (a) $T_{Cu} = 0.005$ M, $T_L = 0.025$ M; (b) $T_{Cu} = 0.005$ M, $T_L = 0.100$ M.

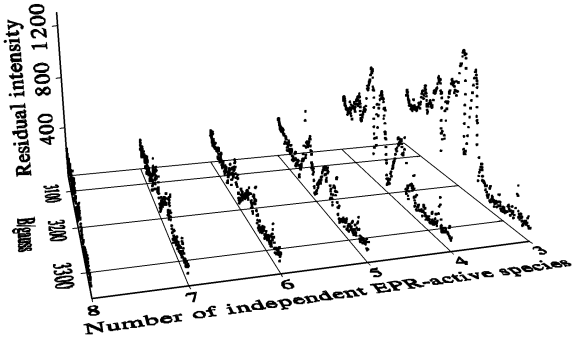

Figure 3. Residual intensity curves obtained by matrix rank analysis for the copper(II)−3-amino-4,4-dimethylpentanoic acid (R = *t*Bu) system for different numbers of EPR-active species.

suggest the reliability of the formation constants; the corresponding overall formation constants determined by pH potentiometry or EPR spectroscopy (Table 1) are in good agreement.

Verification of the Equilibrium Model. Confidence Level of Parameters. For the ligand with R = *t*Bu as an illustrative example, we demonstrate that the decrease in the overall regression coefficient (characterizing the spectral fit for the whole series of spectra) considerably exceeds the critical value if any of the species involved in the best model are omitted (Table 2), which is an indication of the significant difference between the best and the reduced models.

Table 2. Impairment of the Overall Regression Coefficient in the Copper(II)−3-Amino-4,4-dimethylpentanoic Acid (R = *t*Bu) System When One or Another of the Species Implicated in the Best Model Is Omitted[a]

omitted species	ΔR_{actual}
$[CuLH]^{2+}$	0.001 522
$[CuL_2H_2]^{2+}$	0.000 648
$[CuL_2H]^{+}$	0.011 547
$[CuL_2]$, one of the isomers	0.000 363

[a] $R = 0.995\,608$, $\Delta R_{critical} = 0.000\,019$.

The EPR parameters for the various species, together with their confidence intervals 3σ at a significance level of 99.7%, are listed in Table 3, while the component spectra calculated from these and the line width data are depicted in Figure 4.

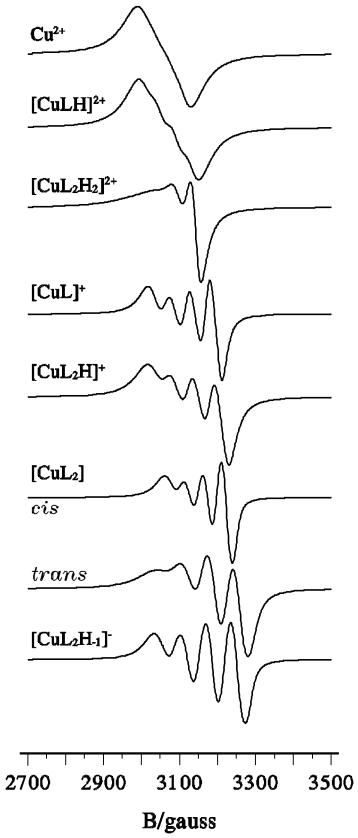

Figure 4. EPR spectra for the various copper(II)−β-alanine complexes, calculated at a frequency of 9.4 GHz from the data in Table 3 (and the relaxation parameters given as Supporting Information).

On the whole, the confidence intervals for g_0 and A_0 are narrow, showing the high reliability of these parameters. A slightly reduced level of confidence was found in those cases where the copper hyperfine splitting of the component spectra is not well-resolved (aqua complex, $[CuLH]^{2+}$, and $[CuL_2H_2]^{2+}$) or the species is formed in low concentration ($[CuL_2H_2]^{2+}$). The N superhyperfine coupling constants a_{N_o} could also be determined with good confidence in most cases, except for the minor isomer of $[CuL_2]$ of a few ligands (Table 3), where this species was formed only in low concentration because of the particularly poor solubility of $[CuL_2]$ and precipitation far from its predominance.

Table 3. EPR Parameters for the Copper(II) Complexes of the Various β-Substituted β-Amino Acids[a]

		species							
R	param	Cu^{2+}	$[CuLH]^{2+}$	$[CuL_2H_2]^{2+}$	$[CuL]^{+}$	$[CuL_2H]^{+}$	*cis*-$[CuL_2]$	*trans*-$[CuL_2]$	$[CuLH_{-1}]^{-}$
H	g_0	2.1960(4)	2.1871(7)	2.1820(9)	2.1554(3)	2.1495(9)	2.1302(1)	2.1247(6)	2.1287(8)
	A_0	34.0(4)	39.4(7)	41.7(16)	51.3(3)	55.0(9)	46.4(1)	62.0(9)	63.0(14)
	a_{N_o}				10.6(4)	12.6(12)	8.8(1)	11.9(6)	11.9(8)
							8.8(1)	11.9(6)	11.9(8)
Me	g_0	2.1981(5)	2.1921(6)	2.1841(10)	2.1549(2)	2.1523(10)	2.1299(1)	2.1289(6)	
	A_0	33.8(5)	42.2(7)	42.7(14)	52.9(2)	57.1(12)	50.5(1)	64.5(7)	
	a_{N_o}				10.1(3)	11.8(15)	8.7(1)	13.6(3)	
							8.7(1)	13.6(3)	
Et	g_0	2.1963(4)	2.1845(5)	2.1905(6)	2.1538(2)	2.1429(5)	2.1284(1)	2.1230(4)	
	A_0	33.7(4)	39.9(5)	48.0(14)	53.4(2)	54.5(6)	52.4(1)	59.5(5)	
	a_{N_o}				9.3(3)	9.0(23)	8.3(1)	13.5(3)	
							8.3(1)	13.5(3)	
*i*Bu	g_0	2.1965(4)	2.1858(7)	2.1850(13)	2.1541(1)	2.1502(5)	2.1282(2)	2.1253(14)	
	A_0	34.3(4)	40.5(5)	44.2(12)	53.5(1)	58.0(6)	51.0(2)	64.8(6)	
	a_{N_o}				10.0(2)	11.1(8)	8.4(2)	7.0(23)	
							8.4(2)	7.0(23)	
*i*Pr	g_0	2.1978(3)	2.1898(5)	2.1759(10)	2.1557(1)	2.1495(7)	2.1306(1)	2.1279(5)	
	A_0	34.0(4)	42.0(6)	34.4(14)	53.8(1)	57.0(7)	53.3(1)	66.4(5)	
	a_{N_o}				10.1(1)	12.2(9)	8.4(1)	12.1(3)	
							8.4(1)	12.1(3)	
*c*Hex	g_0	2.1962(2)	2.1848(3)	2.1779(10)	2.1541(1)	2.1529(7)	2.1246(14)	2.1259(44)	
	A_0	33.8(2)	40.2(2)	41.0(12)	53.8(1)	64.5(6)	47.6(17)	69.7(172)	
	a_{N_o}				9.9(2)	12.9(9)	9.0(12)	13.0(70)	
							9.0(12)	13.0(70)	
1-EtPr	g_0	2.1967(2)	2.1850(4)	2.1843(5)	2.1547(1)	2.1512(4)	2.1221(15)	2.1201(28)	
	A_0	34.0(2)	40.2(3)	43.9(8)	53.5(1)	57.8(4)	43.9(16)	66.1(107)	
	a_{N_o}				9.9(1)	9.8(7)	8.4(14)	5.6(16)	
							8.4(14)	5.6(16)	
*t*Bu	g_0	2.1964(4)	2.1854(4)	2.1776(7)	2.1559(1)	2.1494(1)	2.1244(2)	2.1273(11)	
	A_0	34.6(3)	39.7(3)	37.8(6)	54.7(1)	56.0(1)	50.3(2)	66.1(12)	
	a_{N_o}				9.6(1)	9.1(2)	8.7(1)	6.0(27)	
							8.7(1)	6.0(27)	

[a] The confidence intervals of the parameters regarding the last digit(s) are given in parentheses. The hyperfine coupling constants refer to the isotope ^{63}Cu.

EPR Parameters and Coordination Modes. The general considerations concerning the relation of the coordination modes and EPR parameters have been given elsewhere.[4,7] However, the following is noteworthy: (1) As the ground state of the copper(II) ion in these complexes is $d_{x^2-y^2}$, the EPR parameters are sensitive to alterations in the equatorial coordination, while the axial coordination has only a slight and indirect effect. (2) As the equatorial water molecules are displaced by donor groups with a stronger ligand field, g_0 decreases; the amino N is a stronger donor than the carboxylate O, so its coordination reduces g_0 to a higher extent than does ligation of the latter atom. (3) For effective D_{4h} symmetry, a decrease in g_0 is accompanied by an increase in A_0; an unusually low value of A_0 is diagnostic of rhombic distortion.[4,9−12]

According to the above statements, the EPR data show that the expected equatorial donor atom sets are present in these complexes. The g_0 and A_0 values of $[CuLH]^{2+}$ and $[CuL_2H_2]^{2+}$ are close to those of the aqua complex, since the amino groups are protonated, and only the weak O donors of the water molecules and carboxylate group(s) are bound to the metal ion. As a result of the proton loss and coordination of the amino group, in $[CuL]^{+}$ a stronger ligand field is present, as indicated by the smaller g_0 and larger A_0. For $[CuL_2H]^{+}$, also the bidentate equatorial coordination of the ligand L is most probable; the simultaneous ligation of the carboxylate group of the ligand LH is accompanied by a somewhat lower g_0 and higher A_0 (Table 3) as compared to the parameters of the previous species. The copper coupling constant does not indicate rhombic distortion; therefore, the trans position of the coordinated carboxylate O atoms seems probable, with regard to the fact that negatively charged donor groups can induce significant rhombic distortion if they occupy neighboring equatorial sites.[9,10]

The low g_0 values reveal that both amino groups occupy equatorial positions in both isomers of $[CuL_2]$. Accordingly, two kinds of isomerism may occur: (1) Bidentate equatorial ligation of both ligands takes place in both isomers; then the donor groups of the same kind can be bound in either the cis or the trans position. (2) In the first isomer, diequatorial coordination occurs for both ligands, while, in the second isomer, one of the ligands is bound equatorially by the amino group and axially by the carboxylate group. In the first case, nearly identical g_0 values are to be expected, while, in the second event, the lack of an equatorial carboxylate group is expected to increase the g_0 value of the respective isomer by 0.007−0.01.[7,10] The nearly equal g_0 values for most systems (Table 3) support cis−trans

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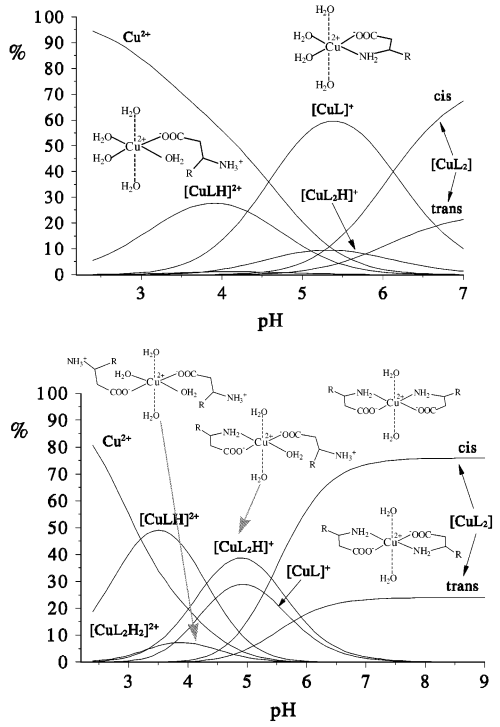

Figure 5. Concentration distribution in the copper(II)−3-amino-4-methylpentanoic acid (R = *i*Pr) system, calculated from the EPR spectroscopic formation constants at T_{Cu}= 0.005 M and T_L = 0.025 M (top) and T_{Cu} = 0.005 M and T_L = 0.100 M (bottom), together with the coordination modes proposed for the various complexes.

isomerism, similarly to $[CuL_2]$ of α-amino acids.[11,12] (For β-alanine and its β-ethyl-substituted derivative, even the difference of ca. 0.005 in the isomers' g_0 values cannot be regarded a decisive evidence for the second type of isomerism.) The low A_0 for the spectrum with larger intensity suggests a significant rhombic distortion, and therefore, the major component curve was assigned to the cis isomer with lower symmetry. For those systems where a_{N_0} could be obtained with good confidence for both microspecies, we observed a smaller N coupling constant for the cis isomer than for the trans isomer (Table 3). That is, distorted geometry disfavors the electron delocalization from the copper(II) $d_{x^2-y^2}$ orbital to the N orbitals of appropriate symmetry. As the Cu−N in-plane σ-bonds become less covalent, more covalent σ-bonds with the O donors and enhanced covalency of π-bonds should contribute to the stability of the (major) cis isomer. The suggested coordination modes and the distribution of copper(II) among the corresponding species are represented in Figure 5.

Effects of Substituents on Complex Equilibria. Farkas and co-workers[13] explain the effects of various substituents on the complex stabilities and on the protonation−deprotonation equilibria of the ligand in terms of a combination of electronic, resonance, and steric contributions, as it is usual. For the present ligands, the resonance effects are negligible, and we have to take into consideration only electronic and steric effects. Taft's σ* constants[14] characterizing the electronic effects of substituents are +0.49, 0, −0.10, −0.125, −0.19, and −0.30 for H and groups Me, Et, *i*Bu, *i*Pr, and *t*Bu, respectively. These effects are expected to influence primarily the basicities (protonation constants) of the amino groups of the ligands, as the amino groups are bound to the same atom of the backbone as the substituents. Accordingly, a slight increase in log K in the above order of increasing electron-releasing effect of the side chain would be expected in the series of ligands. In contrast, the formation constants of the proton complexes [HL] (Table 1) do not exhibit the expected trend: There are insignificant differences for the majority of ligands; the only exceptions are the R = *i*Pr and *t*Bu derivatives of slightly lower protonation constants. The most probable explanation for this is the increasing steric hindrance of substituents (see below) which counterbalances, moreover, for the ligands with R = *i*Pr and *t*Bu, surpasses their electron-releasing effect.

The steric effects of substituents can be characterized either by Taft's experimental parameter E_S,[14] derived primarily from kinetic studies, or by parameters calculated from the molecular geometry. One of the latter is the volume v obtained from the van der Waals radius of the substituent, introduced by Charton,[15] whose approach was refined by Meyer.[16] According to Meyer's concept, the steric hindrance is exerted mainly near the reaction center, by a limited portion of the substituent. The corresponding parameter V^a is the volume occupied by the substituent within a sphere of radius 0.3 nm around the anchor atom (i.e. the atom connected to the backbone). It is obtained from molecular mechanics calculations and increases in the sequence H, Me, Et, *i*Bu, *i*Pr, *c*Hex, 1-EtPr, and *t*Bu (V^a =0, 2.84, 4.31, 5.26, 5.74, 6.25, 6.67, and 7.16 in 1×10^{-2} nm^3/molecule units,[16] respectively).

The formation constants of the various species themselves (Table 1) do not correlate with the steric parameter in the present series. In contrast, the substituents exert significant and systematic effects on the interconversions of the protonated complexes $[CuLH]^{2+}$ and $[CuL_2H]^+$ and the corresponding nonprotonated ones $[CuL]^+$ and $[CuL_2]$. The equilibrium constants $pK = -\log K = \log \beta_{\text{protonated complex}} - \log \beta_{\text{nonprotonated complex}}$ for the deprotonation process and log K for the corresponding protonation process (which are equal to each other and are obtained from the data in Table 1) decrease with increasing steric parameters. The good linear correlations between the protonation log K values and V^a are depicted in Figure 6. (The correlation with E_s or v was found to be somewhat poorer, suggesting that the volume occupied by the side chain around the anchor atom, i.e., near the amino group, makes the predominant contribution.) These correlations reveal that the formation of the nonprotonated complexes is shifted to lower pH as more and more bulky substituents are present; in other words, at a given pH, the nonprotonated complexes are more favored for ligands with substituents of higher V^a.

We can explain the above correlation by the significantly different efficiencies of steric shielding in the protonated and

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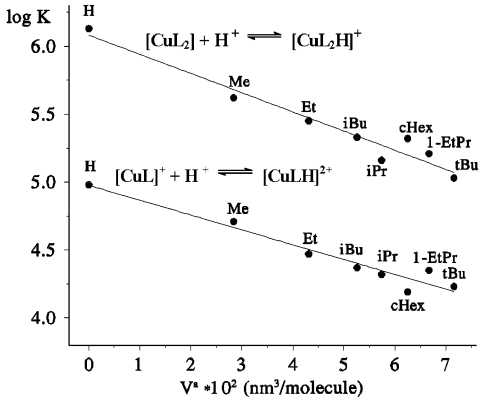

Figure 6. Correlation between the protonation constants as log *K* of the complexes $[CuL_2]$ and $[CuL]^+$ and the steric parameter V^a. The correlation coefficients obtained from the linear regression analysis are 0.9754 and 0.9720 for the top and the bottom lines, respectively.

nonprotonated species. To understand the reason for this, we have to take into consideration the structural changes accompanied by the protonation−deprotonation taking place on an amino group. The NH_3^+ group in $[CuLH]^{2+}$ and $[CuL_2H]^+$ can move away the first coordination sphere almost unimpeded, and the proton loss can proceed easily in the bulk solution. In contrast, once the NH_2 group has been bound to the metal ion in $[CuL]^+$ or $[CuL_2]$, the first coordination sphere shields it from the attack of protons in the bulk solution, blocking certain directions. This in turn amplifies the importance of the steric hindrance of the substituents in the nonprotonated complexes: A bulky substituent hinders the protonation of $[CuL]^+$ or $[CuL_2]$ to a higher extent than a smaller one does, and since the effect of the side chain on the proton loss of $[CuLH]^{2+}$ or $[CuL_2H]^+$ does not vary significantly in the series, the equilibrium of protonated versus nonprotonated species is shifted in favor of the latter to an increasing extent as the steric effect of the substituent increases.

Conclusions

The two-dimensional EPR simulation method allows a more detailed analysis of the equilibria in the copper(II)−*β*-substituted *β*-amino acid systems; besides the major species $[CuL]^+$ and $[CuL_2]$, also identified by pH potentiometry, we have demonstrated 3 protonated complexes ($[CuLH]^{2+}$, $[CuL_2H_2]^{2+}$, and $[CuL_2H]^+$) and cis−trans isomerism for $[CuL_2]$. We have found that (1) the interconversion processes between $[CuL]^+$ and $[CuLH]^{2+}$ and between $[CuL_2]$ and $[CuL_2H]^+$ are controlled primarily by the steric effects of the alkyl (and cycloalkyl) side chains, (2) the protonation is hindered to a higher extent than the proton loss, and (3) in comparison with small side chains, bulky substituents favor the corresponding nonprotonated species.

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Supporting Information Available: A complete set of EPR parameters for all complexes, figures that illustrate the impairment of spectral fit at various ligand concentrations and pH's, when neglecting one or other complex implicated in the best model. This material is available free of charge via the Internet at http://pubs.acs.org.

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