Composition, Stability, and Lability of Copper(II) Dipeptide Complexes

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Complex formation of copper(II) with glycylglycine (HL) has been studied spectrophotometrically over a wide range of ligand concentration (0.005—1.0 mol dm^{-3}) and pH (0.5—13) in aqueous potassium nitrate (1.0 mol dm^{-3} KNO_3). The complexes $[CuL]^+$, $[Cu(HL)]^{2+}$, $[Cu(LH_{-1})]$, $[Cu(LH_{-1})(OH)]^-$, $[Cu(LH_{-1})L]^-$, $[Cu(LH_{-1})_2]^{2-}$, and $[Cu_2(LH_{-1})_2(OH)]^-$ were found and their formation constants determined. The ligand in $[Cu(HL)]^{2+}$ is co-ordinated through the carboxylate group, and the terminal amino group remains protonated. Such a co-ordination mode is more favourable for depeptides as compared to amino acids. Both of the ligands in $[Cu(LH_{-1})_2]^{2-}$ are bound to the metal ion in a bidentate mode through the terminal amino nitrogen and the deprotonated peptide nitrogen. The species $[Cu_2(LH_{-1})_2(OH)]^-$ has an intense absorption band in the near-u.v. region which indicates that the OH^- group is bridging. The lability of complexes $[Cu(LH_{-1})L]^-$ with five aliphatic dipeptides has been investigated by n.m.r. relaxation of water protons. The ligand-exchange rates (R_{ex}) in solutions of these complexes follow the kinetic equation $R_{ex} = (k_1 + k_2[L^-])[Cu(LH_{-1})L^-]$. Increasing the size of the side chain of the dipeptides leads to a lower R_{ex}, and a good correlation between log k_2 and the steric constants E_s^0 of the side-chain substituents is observed. The high values for k_1 obtained indicate a considerable *trans* effect of the deprotonated peptide group.

Attention has been paid to copper(II) oligopeptide complexes first of all as models for the active sites of copper-containing enzymes. For the understanding of the mechanisms of enzyme action, a study of co-ordinated ligand mobility in the model compounds is of particular importance.

Among other copper(II) dipeptide complexes, the species with glycylglycine (HL) have been investigated extensively. These studies[1-24] have clearly shown the existence of the copper(II) complexes $[CuL]^+$, $[Cu(LH_{-1})]$, $[Cu(LH_{-1})(OH)]^-$, and $[Cu(LH_{-1})L]^-$ where LH_{-1}^{2-} represents the amide deprotonated form of the ligand. Besides these species, other complexes $[Cu(LH_{-1})(OH)_2]^{2-}$, $[Cu(HL)]^{2+}$, $[CuL_2]$ $[Cu(LH_{-1})_2]^{2-}$, and $[Cu_2(LH_{-1})_2(OH)]^-$ have been proposed,[2,5,8,11,12,15,17,18,20,22,23] but rigorous evidence for their presence has not been provided. In this connection, it is necessary to extend the range of ligand concentrations and pH in order to accumulate and identify the species which could be minor components under ordinary conditions ($c_L \leqslant 0.05$ mol dm^{-3}, pH 3—11). In the present work we have attempted such investigations by means of a spectrophotometric method.

The exchange of nitrogen-containing ligands co-ordinated to copper(II) may be studied by n.m.r. relaxation of water protons.[25,26] Previous investigations[22,24,27] of the copper(II)–glycylglycine system by this method have given different results. It was assumed[22,24] that the ligand exchange between the first co-ordination sphere of $[Cu(LH_{-1})L]^-$ and the bulk of the solution can be described by a second-order kinetic equation. However, further investigation[27] has shown that the rate law for the exchange process includes both a second-order and a first-order term, as in the case of the mixed-ligand complex $[Cu(LH_{-1})L']$ with L′ = ethanolamine.[28] To solve the above problem, we have studied the lability of copper(II) complexes with a series of the dipeptides which differ in the alkyl substituents of the side chain. Also, this allows one to estimate a steric effect in the ligand-exchange reactions.

Experimental

Copper(II) nitrate (chemical pure, Reakhim), glycylglycine (analytical grade, Serva), glycyl-DL-α-alanine, glycyl-DL-norvaline, glycyl-DL-valine, and glycyl-DL-leucine (Reanal) were used. The concentration of the copper stock solution was examined by iodometric titration. Each solution contained 1.0 mol dm^{-3} KNO_3 (chemical pure, Reakhim). The pH was adjusted to the desired value with solutions of HNO_3 and KOH. The temperature of the solutions was kept constant within 1 K.

The parameters measured were the pH, optical density, and the water-proton transverse relaxation time (T_2). The pH values were determined on a pH-meter pH-673 with an error of ±0.01 pH unit. The visible absorption spectra were recorded with Hitachi 557 and SF 18 spectrophotometers having a reproducibility of ±1%. The T_2 measurements were carried out with an accuracy of ±2% using a NMR Specialties pulsed coherent spectrometer operating at 15 MHz by the Carr–Purcell–Meiboom–Gill method.[25]

The composition and formation constants of the complexes were calculated from the pH dependences of the molar absorption coefficient (ε) at several metal:ligand ratios (T = 293 K). Rate constants of the ligand-exchange reactions were obtained from the dependences of the molar relaxation coefficient[25] $1/c_M T_{2p}$ upon the concentrations of the dipeptides at fixed pH values (T = 298 K). Protonation constants pK_2 for all the five dipeptides at 298 K as well as pK_1 and pK_2 for glycylglycine at 293 K were needed for the calculations and were determined from the potentiometric titration curves in the absence of metal ion.

All calculations were made on an Elektronika D3-28 microcomputer with the aid of the CPESSP computer program.[29] The program is based on the minimization of a function F[30] [equation (1)] where $x_{exptl.,j}$ and $x_{calc.,j}$ are the experimental

$$F = \sum_{j=1}^{N} (x_{exptl.,j} - x_{calc.,j})^2 \frac{1}{\sigma^2 x_{exptl.,j}^2} \qquad (1)$$

and calculated properties, respectively, j is the experiment number (N experiments), and σ is the relative error of the measurement. The reliability of the results obtained with this procedure was evaluated by the Fisher criterion using the computed $F_{min.}$ value and the experimental error as described.[30]

Table 1. Composition, formation constants, and absorption spectral parameters of the copper(II) complexes with glycylglycine (298 K, 1.0 mol dm^{-3} KNO_3)

Composition	$\log \beta_{pqr}$	$\lambda_{max.} \pm 5$/ nm	$\varepsilon_{max.}$/ $dm^3\ mol^{-1}\ cm^{-1}$
$[Cu(HL)]^{2+}$	9.36 ± 0.08	770	33
$[CuL]^{+}$	5.63 ± 0.03	750	56
$[Cu(LH_{-1})]$	1.24 ± 0.03	640	82
$[Cu(LH_{-1})(OH)]^{-}$	−8.28 ± 0.02	645	75
$[Cu(LH_{-1})L]^{-}$	4.34 ± 0.07	620	85
$[Cu(LH_{-1})_2]^{2-}$	−7.70 ± 0.07	535	78
$[Cu_2(LH_{-1})_2(OH)]^{-}$	−4.65 ± 0.03	650	82
		326	190

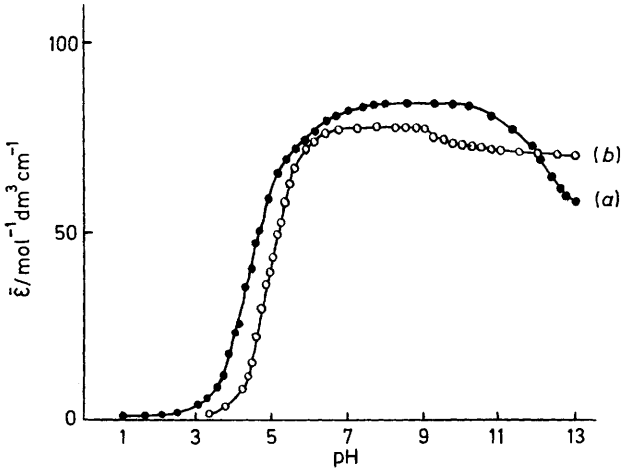

Figure 1. pH Dependence of the absorption coefficient in the copper(II)–glycylglycine system at $c_L = 5.2 \times 10^{-3}$ mol dm^{-3} (*a*) and 0.10 mol dm^{-3} (*b*), $c_M = 5.0 \times 10^{-3}$ mol dm^{-3}, $\lambda = 630$ nm, and $T = 293$ K

Results and Discussion

Composition and Stability.—Figure 1 shows the pH dependence of the molar absorption coefficient in the copper(II)–glycylglycine system at 1:1 (*a*) and 1:20 (*b*) metal:ligand ratios. For the calculation of the complex-formation constants from these curves the values of $pK_1 = 3.24 \pm 0.02$ and $pK_2 = 8.32 \pm 0.02$ for glycylglycine obtained at 293 K were used. The formation constant of the complex $Cu_pL_qH_r$ $^{2p-q+r}$ was expressed by $\beta_{pqr} = [Cu_pL_qH_r]^{2p-q+r}/[Cu^{2+}]^p[L^-]^q[H^+]^r$ and the corresponding molar absorption coefficient was denoted as ε_{pqr}. From the (*a*) series in the pH region below 7, β_{11-1} can be computed with high accuracy ($\log \beta_{11-1} = 1.24 \pm 0.03$), but the calculated value of β_{110} is uncertain. The use of the above β_{11-1} value in the data treatment of the (*b*) series at pH ≤ 9 allows a reliable β_{12-1} value to be determined. However, it was impossible to attain a good fit of the experimental data within the range pH 1—5 at a 1:20 metal:ligand ratio taking into account only the species $[Cu(LH_{-1})]$, *i.e.* at least $[CuL]^+$ was also present under these conditions. Using species $[CuL]^+$, $[Cu(LH_{-1})]$, and $[Cu(LH_{-1})L]^-$ for the (*b*) curve simulation (pH 0.5—9), one obtains β_{110} and ε_{110} values which prevent a satisfactory description of the (*a*) series. A full convergence in the simulation of the (*a*) and (*b*) series data resulted only upon addition of $[Cu(HL)]^{2+}$ to above three species. The formation of this complex is more obvious from the spectrophotometric data at high glycylglycine concentration. For example, without consideration of $[Cu(HL)]^{2+}$, the high value of the average molar absorption coefficient $\varepsilon = 27$ $dm^3\ mol^{-1}\ cm^{-1}$ at 770 nm ($c_L = 1.0$ mol dm^{-3}, pH 2.30) cannot be satisfactorily explained because under these conditions the accumulation of $[CuL]^+$ does not exceed 5% and for Cu^{2+} $\varepsilon = 11$ $dm^3\ mol^{-1}\ cm^{-1}$ at 770 nm. The derived formation constants and absorption spectral parameters of the four complexes are given in Table 1.

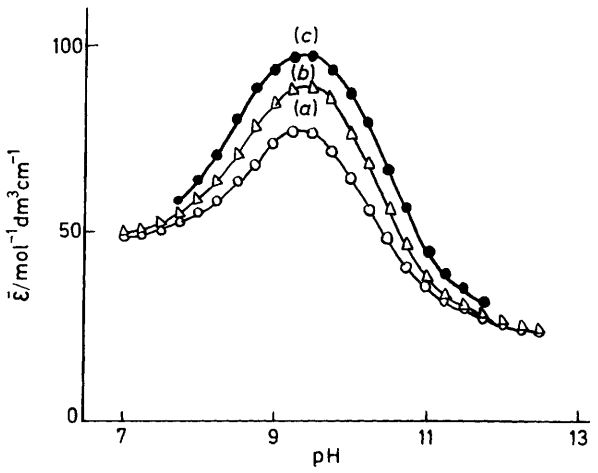

Figure 2. pH Dependence of the absorption coefficient in the copper(II)–glycylglycine system at a 1:1 metal:ligand ratio, $c_M = 5.0 \times 10^{-3}$ (*a*), 9.8×10^{-3} (*b*), and 1.5×10^{-2} mol dm^{-3} (*c*); $\lambda = 330$ nm, $T = 293$ K

Let us consider the alkaline region in Figure 1. With increasing pH from 9 to 13 ε is slightly decreased in the (*b*) series and significantly reduced in the (*a*) series. Moreover, at a metal:ligand ratio of 1:1 some variation of ε *vs.* total metal concentration in this pH range was observed but it was not so large as to warrant further consideration. At the same time in the near-u.v. region a strong dependence of ε upon metal concentration and pH was revealed (Figure 2). The simulation of curves (*a*)—(*c*) clearly indicates formation of the species $[Cu(LH_{-1})(OH)]^-$ and $[Cu_2(LH_{-1})_2(OH)]^-$, and the maximum accumulation of the latter was reached at pH ≈ 9.5. The values of the stability constants and absorption spectral parameters of the hydroxy-complexes are listed in Table 1. As can be seen from Figure 1 and Table 1, the visible absorption spectrum of $[Cu(LH_{-1})(OH)]^-$ is very similar to that of $[Cu(LH_{-1})L]^-$. Therefore, the mentioned ε reduction in the (*b*) series does not arise from the replacement of the L^- ligand co-ordinated in the $[Cu(LH_{-1})L]^-$ complex by the OH^- ion but from further deprotonation of this species at pH ≈ 9 (Figure 5). The simultaneous existence of both processes is possible but the latter should dominate at high ligand concentrations. Such a situation is realized at $c_L = 1.0$ mol dm^{-3} (Figure 3). However, the approximate isosbestic point at 575 nm (Figure 3) provides no evidence for the presence in equilibria of only two forms, $[Cu(LH_{-1})L]^-$ and $[Cu(LH_{-1})_2]^{2-}$, because of the mentioned similarity of the first species spectrum to that of the third likely complex, $[Cu(LH_{-1})(OH)]^-$. Taking into consideration the three above species, the formation constant and spectral characteristics of the complex $[Cu(LH_{-1})_2]^{2-}$ were calculated from the data in Figure 3 (Table 1). The species distribution diagrams are shown in Figures 4 and 5.

The $\log \beta_{110}$, $\log \beta_{11-1}$, and $\log \beta_{12-1}$ values obtained (Table 1) are closely similar to those reported earlier[2,3,5–10,12–15,17–23] at 293 or 298 K and ionic strengths from 0.1 to 1.0 mol dm^{-3}. The disputable complexes $[Cu(HL)]^{2+}$ and $[Cu(LH_{-1})_2]^{2-}$ were unambiguously identified in solution. The published values of $\log \beta_{111} = 8.14$[17] and $\log \beta_{12-2} = -5.31$,[2] -5.20,[5] and -5.26[20] appear mistaken since these species cannot accumulate to significant extents at

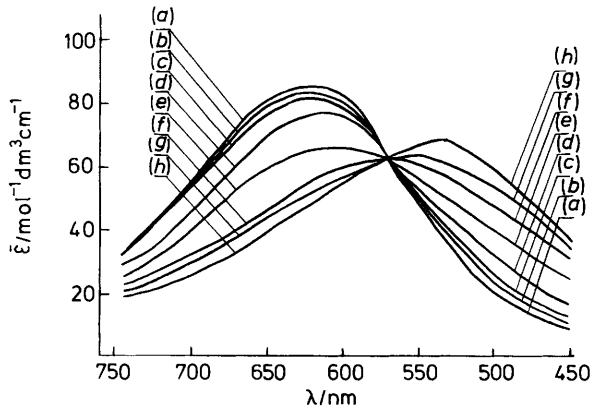

Figure 3. Electronic absorption spectra of the copper(II)–glycylglycine system at c_L = 1.0 mol dm^{-3}, c_M = 5.0 × 10^{-3} mol dm^{-3}, T = 293 K, and various pH: 9.83 (*a*), 10.52 (*b*), 11.00 (*c*), 11.60 (*d*), 12.05 (*e*), 12.50 (*f*), 12.85 (*g*), and 13.27 (*h*)

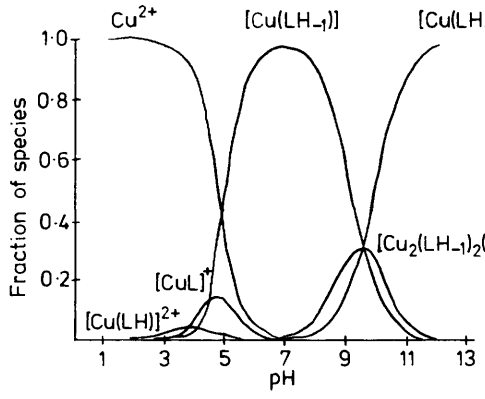

Figure 4. Dependence of the species distribution on pH for the copper(II)–glycylglycine system at a 1:1 metal:ligand ratio, c_M = 5.0 × 10^{-3} mol dm^{-3}

the low ligand concentrations used in the investigations.[2,5,17,20] The agreement between our log β_{12-2} value and that from another investigation[11] carried out at high glycylglycine concentrations (log β_{12-2} = −7.29, I = 1.0 mol dm^{-3}, NaCl) is relatively good. However, for the complex $[Cu(LH_{-1})_2]^{2-}$ the $\varepsilon_{max.}$ value obtained is somewhat larger than those previously reported.[11,31] This may probably be explained by the incomplete account taken of the species coexisting with $[Cu(LH_{-1})_2]^{2-}$ under the experimental conditions of the earlier studies.[11,31]

The ligand in the complex $[Cu(HL)]^{2+}$ is co-ordinated through the carboxylate group (not excepting weak co-ordination of the adjacent peptide carbonyl group) and the additional proton of $[Cu(HL)]^{2+}$ remains attached to the terminal amino nitrogen. This is evident from the higher pK_a value for $[Cu(HL)]^{2+}$ (pK = log β_{111} − log β_{110} = 3.73) than for H_2L^+ (pK_1 = 3.24) and from the larger $\lambda_{max.}$ value of the species $[Cu(HL)]^{2+}$ in comparison with $[CuL]^+$ (Table 1) in which, as is known, the ligand is bound to the metal ion by means of the terminal amino group and the adjacent peptide oxygen. Co-ordination in the zwitterionic form (HL) is more favoured for dipeptides as compared to amino acids. Intramolecular hydrogen bonding between the $-NH_3^+$ group and the $-CO_2^-$ group of the zwitterion (cyclization) is more advantageous for amino acids owing to the steric requirements. For this reason, amino acids have lower pK_1 values and higher

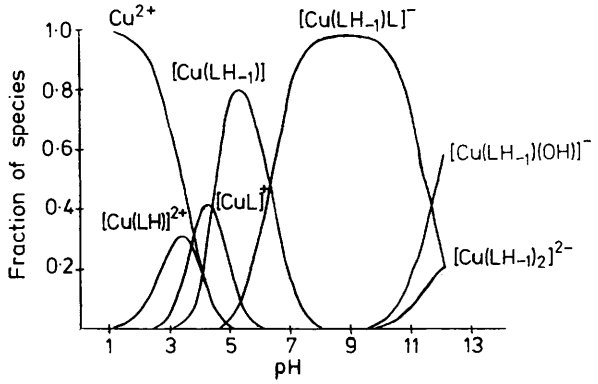

Figure 5. Species distribution as a function of pH for the copper(II)–glycylglycine system at a 1:20 metal:ligand ratio, c_M = 5.0 × 10^{-3} mol dm^{-3}

pK_2 values: *cf.* pK_1 = 3.24, pK_2 = 8.24 for glycylglycine and pK_1 = 2.35, pK_2 = 9.78 for glycine.[32] Thus, the zwitterionic forms of amino acids are less favoured than in the case of dipeptides.

The large $\lambda_{max.}$ shift towards shorter wavelengths on going from $[Cu(LH_{-1})L]^-$ to $[Cu(LH_{-1})_2]^{2-}$ may be explained only by assuming deprotonation of the second peptide nitrogen, because even the replacement of the co-ordinated water molecule by the hydroxide ion resulted in no blue shift of the visible absorption maximum as shown above. Therefore, both ligands LH_{-1}^{2-} in $[Cu(LH_{-1})_2]^{2-}$ are co-ordinated to the metal ion through the terminal amino nitrogen and the deprotonated peptide nitrogen as proposed previously.[11,31] Such a co-ordination mode was found earlier in the crystal structure of dipotassium bis(glycylglycinato)cuprate(II) hexahydrate.[33] Bidentate co-ordination of a dipeptide has been suggested also for aqueous solutions of the mixed-ligand complexes $[Cu(LH_{-1})Q]^-$ with Q^- = amino acidate.[27]

In the complex $[Cu_2(LH_{-1})_2(OH)]^-$ the metal–metal interaction is indicated by the intense absorption band in the near-u.v. region ($\lambda_{max.}$ 326 nm). Monomeric species did not show this band. An analogous absorption band for tetra-ammine-di-μ-hydroxo-dicopper(II) was detected.[34,35] It was assigned[34,36] to charge transfer from bridging oxygen p_π orbitals to the hole in $d_{x^2-y^2}$ orbital of copper. Therefore, the OH^- group in $[Cu_2(LH_{-1})_2(OH)]^-$ is bridging.

Ligand Exchange.—The results of the present investigation show that the $[Cu(LH_{-1})L]^-$ species dominates in the copper(II)–glycylglycine system at pH ≈ 8 and $[L^-] \geqslant 0.01$ mol dm^{-3}. This holds true also for other aliphatic dipeptides, as can be ascertained from a comparison of their stability constants,[32] and allows a study of the ligand-exchange reactions in solutions of copper(II) dipeptide complexes $[Cu(LH_{-1})L]^-$.

Figures 6 and 7 show the ligand-concentration dependence of the parameter $1/c_M T_{2p}$ ($1/T_{2p}$ is the paramagnetic contribution of the transverse relaxation rate) in solutions of copper(II) complexes with five dipeptides. For each system, equation (2) is

$$\frac{1}{c_M T_{2p}} = \frac{P'_A}{\tau_M^{(A)} + T_{2M}^{(A)}} + \frac{P'_E}{\tau_M^{(E)} + T_{2M}^{(E)}} = K_A + \frac{P'_E}{(k_1 + k_2[L^-])^{-1} + T_{2M}^{(E)}} \quad (2)$$

obeyed which is a special case of the Swift–Connick equation.[37] In this expression A and E refer to the exchangeable protons of the axially and equatorially bonded ligands, respectively, H_2O and L^-, $P' = P_M/c_M$, P_M is the mole fraction of the bound

Table 2. Proton relaxation parameters and ligand L^- exchange rate constants in solutions of the complexes $[Cu(LH_{-1})L]^-$ (HL = $H_2NCH_2CONHCHRCO_2H$, 298 K, 1.0 mol dm^{-3} KNO_3)

Dipeptide	R	$10^{-6}k_2$/dm^3 mol^{-1} s^{-1}	$10^{-5}k_1$/s^{-1}	10^7T_{2M}/s	E_s^0(R)	$pK_2 \pm 0.02$
Glycylglycine	H	14.5 ± 0.7	1.7 ± 0.7	8.5 ± 0.5	0.25	8.24
Glycyl-DL-α-alanine	Me	12.3 ± 0.3	0.8 ± 0.3	8.4 ± 0.2	0	8.28
Glycyl-DL-norvaline	Pr^n	9.2 ± 0.2	1.0 ± 0.2	8.5 ± 0.2	−0.56	8.33
Glycyl-DL-valine	Pr^i	8.0 ± 0.2	0.8 ± 0.2	8.5 ± 0.3	−0.85	8.34
Glycyl-DL-leucine	Bu^i	6.3 ± 0.1	0.7 ± 0.3	8.5 ± 0.2	−1.13	8.29

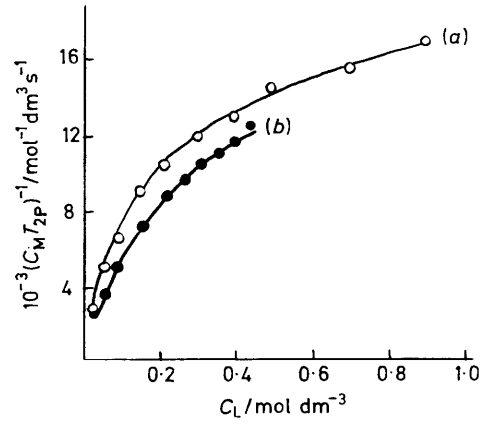

Figure 6. Ligand-concentration dependence of the parameter $1/c_MT_{2p}$ in the copper(II)–glycylglycine system at pH 7.9 (*a*) and in the copper(II)–glycyl-DL-norvaline system at pH 8.1 (*b*), $c_M = 6.5 \times 10^{-3}$ mol dm^{-3}, and $T = 298$ K

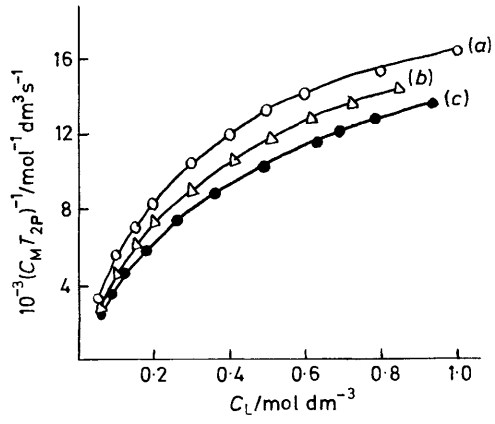

Figure 7. Dependence of the parameter $1/c_MT_{2p}$ in the copper(II)–dipeptide system on the concentrations of glycyl-DL-α-alanine (*a*), glycyl-DL-valine (*b*), and glycyl-DL-leucine (*c*) at pH 7.9 [(*a*), (*b*)] and pH 8.2 [(*c*)], $c_M = 6.5 \times 10^{-3}$ mol dm^{-3}, and $T = 298$ K

protons, τ_M is the residence time of the bound proton, and T_{2M} is its relaxation time. Expression (2) means that the ligand exchange in solutions of the $[Cu(LH_{-1})L]^-$ complexes [equation (3)] follows the kinetic equation (4) with first-order

$$[Cu(LH_{-1})L]^- + L^{*-} \rightleftharpoons [Cu(LH_{-1})L^*]^- + L^- \quad (3)$$

$$R_{ex} = (k_1 + k_2[L^-])[Cu(LH_{-1})L^-] \quad (4)$$

and second-order rate constants, k_1 and k_2. The exchange of the equatorially bonded tridentate ligand LH_{-1}^{2-} was not considered in equation (2) because it was very slow.[28] The value of K_A (the contribution of the axially bonded H_2O molecules to the relaxation rate) does not exceed 300 dm^3 mol^{-1} s^{-1} and may thus be neglected in the data analysis.

In contrast with the above interpretation, the authors of an earlier investigation[22] explained the high value of the ligand-concentration-independent contribution to the molar relaxation coefficient of the $[Cu(LH_{-1})L]^-$ complex as follows. 'There is a continuous intramolecular rearrangement of the ligands in $[Cu(LH_{-1})L]^-$ and during this process one of the $-NH_2$ groups may become free for a short time, which is long enough for the proton exchange with water to take place,' analogous to the copper(II) complexes with diaminomonocarboxylates (ornithine and lysine) studied previously.[38] However, the experimental results[38] relate only to special conditions (1:2 metal:ligand ratio, $c_L = 0.02$—0.06 mol dm^{-3}, pH up to 11) which do not exclude hydroxo-complex formation[39] and lead to the appearance of the free amino acids and their exchange by the second-order process. At a sufficient ligand excess the same authors[40] did not observe any anomalous relaxation effect for the copper(II) complexes of ornithine and lysine as compared to other amino acids. In any case, the short-lived replacement of the equatorially bonded NH_2 group of LH_{-1}^{2-} by the axially bonded peptide carbonyl group of L^- apparently implied[22,38] is very unfavourable in view of the much lower co-ordination ability of the peptide carbonyl oxygen in comparison with the terminal amino nitrogen.

The values of k_1, k_2, and T_{2M} computed by using equation (2) are listed in Table 2. The dipeptide pK_2 values obtained and the E_s^0 constants[41] of the side-chain substituents (R) in the dipeptide ligands are also given.

As expected, the relaxation time T_{2M}, being sensitive only to the immediate environment of the metal ion, was found to be substituent independent. At the same time, k_2 decreases progressively with increasing size of R, and a linear correlation between $\log k_2$ and the steric constant E_s^0 according to the Taft equation[41] (5) is observed with a correlation coefficient $r =$

$$\log k_2 = \log k_2^0 + \delta E_s^0 \quad (5)$$

0.996. The $\log k_2^0$ and δ values and their least-squares standard deviations obtained are $\log k_2^0 = 7.098 \pm 0.009$ and $\delta = 0.251 \pm 0.013$. The presence of the above steric effect in the dipeptide exchange processes confirms the associative-type mechanism of the ligand-substitution reactions of the copper(II) complexes.

The absence of a regular change in k_1 as the size of R increases is not in contradiction with an associative-type mechanism since the water molecules, being small, are able to react as a nucleophile in the k_1 pathway. However the limited accuracy of the k_1 values obtained does not allow one to affirm that a steric effect is completely absent in this process. In any case, the high k_1 values for the dipeptides studied indicate a considerable *trans* effect of the deprotonated peptide group, as previously emphasized.[28] From previous data,[28] and the stability constants of bis(asparaginato)copper(II),[42] bis-

(iminodiacetato)cuprate(II),[43] and the glycylglycinato complex $[Cu(LH_{-1})L]^-$ (present work), one may calculate the stepwise constants for binding of ethanolamine to the above three complexes as $10^{4.44}$, $10^{4.29}$, and $10^{2.77}$ correspondingly. The low magnitude of the latter can be explained by the *trans* effect of the deprotonated peptide nitrogen and accounts for the high rate of ethanolamine dissociation in a first-order process that is not detected for the other two species.

From k_1 and the stepwise stability constant (K_2) of the complex $[Cu(LH_{-1})L]^-$, the forward rate constant (k_f) of reaction (6) may be calculated from the relation $k_f = k_1K_2$. For

$$[Cu(LH_{-1})] + L^- \underset{k_1}{\overset{k_f}{\rightleftharpoons}} [Cu(LH_{-1})L]^- \qquad (6)$$

all the five dipeptides considered the values of both k_1 and K_2 are similar, $\bar{k}_1 = 1 \times 10^5$ s^{-1} (Table 2) and $\bar{K}_2 = 1 \times 10^3$ dm^3 mol^{-1} (Table 1 and ref. 32), and so the average k_f value is $\bar{k}_f \approx 1 \times 10^8$ dm^3 mol^{-1} s^{-1}. It would be of interest to obtain the k_f constants by other relaxation methods.

Acknowledgements

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