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Structure, Stability, and Lability of Copper(II) Complexes with Triglycine

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Abstract — Equilibrium constants of complex formation, rate constants of chemical exchange reactions, and characteristics of electronic absorption spectra for species detected in aqueous solution of copper(II) with triglycine were determined, and conclusions on the structure of the complexes were made. A possibility of H-bond formation between the ammonium group of the zwitter-ionic form of the ligand and the second peptide oxygen in the anionic form of an adjacent ligand was shown. Kinetics and mechanisms of ligand and proton exchanges in solutions of copper(II) bistripeptide complexes with the ligand containing a deprotonated peptide nitrogen atom were studied. A new mechanism was proposed for hydroxide-catalyzed substitution reactions in copper(II) complexes with tripeptides.

Copper(II) complexes with oligopeptides are of considerable interest for coordination and bioinorganic chemistry as models of metal enzymes, mediators, and metal transport forms in living organisms. Equilibria of complex formation between copper(II) and triglycine [1–7] have been studied in rather narrow ranges of metal and ligand concentrations. An exception is the work [4], but the equilibrium constants therein have been determined on a specific background (3 M $NaClO_4$) and are inapplicable in most other calculations. The cited publications contain no data on lability parameters for the detected complexes, and not all their structures have been considered.

In this work equilibrium constants of complex formation, rate constants of chemical exchange reactions, and characteristics of electronic absorption spectra for species in aqueous solution of copper(II) with triglycine were determined by spectrophotometry and nuclear magnetic relaxation in combination with mathematical simulation. On this basis certain conclusions on the structure of the complexes under study were made. Earlier we determined similar parameters for copper(II) compounds with diglycine [8]. The aim of this work was to reveal changes in the structure, stability, and dynamic behavior of copper(II) complexes on transition from di- to triglycine ligands. Preliminary results of this work were reported earlier [9].

Unlike other works, here we studied the copper(II)–triglycine (LH) system within as-wide-as-possible ranges of pH (1–13) and metal (0.003–0.1 M) and ligand (up to 0.5 M) concentrations on the background of a 1 M solution of KNO_3 at 298 K (only regions of precipitate formation were not covered). To minimize hydrolysis of the ligand, we studied freshly prepared solutions whose stability was controlled by the invariance of their optical density from the moment of their preparation. Thus we could obtain reproducible data for alkaline media at a 1 : 1 metal : ligand ratio.

Stability and structure of complexes. Figure 1a exemplifies the pH dependences of molar spin–lattice relaxation coefficients of water protons $(c_M T_{1p})^{-1}$ [10] and of extinction (ε) at two wavelengths (550 and 650 nm) and a 1 : 1 metal : ligand ratio. At this metal : ligand ratio, the above-mentioned parameters depend on the total metal concentration (c_M), which points to formation of polynuclear complexes. As the ligand concentration increases up to 4 or 20 the metal concentration, the pH dependences of optical and relaxation parameters change essentially (Fig. 1b), implying formation of biscomplexes. We were able to simulate adequately all the dependences using the CPESSP program [11], taking into account 13 complexes (an example of the distribution diagram at a 1 : 20 metal : ligand ratio is given in Fig. 2). Based on the calculated accumulation degrees of the complexes (α), we reconstructed from the electronic absorption spectra of solutions of various compositions by means of the Origin 5.0 program the spectra of all the individual forms. Therewith, the self-consistency procedure was used: Equilibrium constants were varied until the ε values at 550 and 650 nm in the reconstructed spectra of each complex coincide within the limits of experimental errors with those calculated from the initial series (Fig. 1). The constants of

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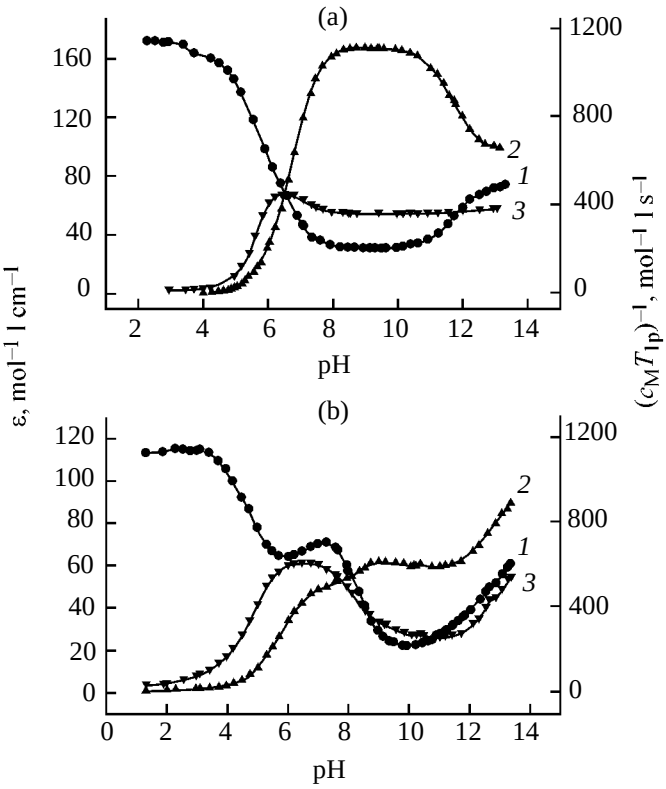


Fig. 1. (*1*) Molar spin–lattice relaxation coefficients $(c_M T_{1p})^{-1}$ and extinction coefficients (ε) at (*2*) 550 and (*3*) 650 nm as functions of pH in the system copper(II)–triglycine ($c_{Cu(II)}$ 4.99×10^{-3} and c_{KNO_3} 1 M, 298 K) for (a) c_L 5.30×10^{-3} and (b) 0.10 M.

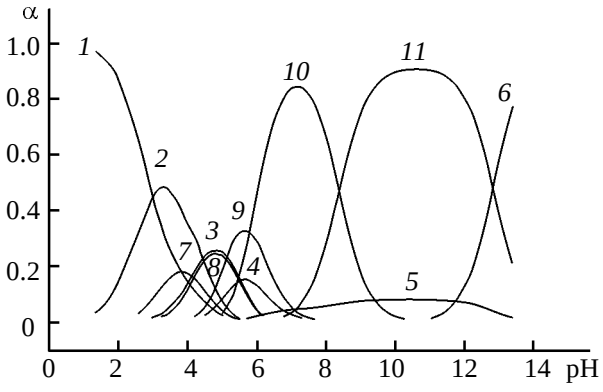


Fig. 2. Distribution of accumulation fractions of complexes (α) as a function of pH in the system copper(II)–triglycine ($c_{Cu(II)}$ 4.99×10^{-3}, c_L 0.10, and c_{KNO_3} 1 M, 298 K). (*1*) Cu^{2+}, (*2*) $CuLH^{2+}$, (*3*) CuL^{+}, (*4*) $CuLH_{-1}$, (*5*) $CuLH_{-2}^{-}$, (*6*) $CuLH_{-3}^{2-}$, (*7*) $CuL_2H_2^{2+}$, (*8*) CuL_2H^{+}, (*9*) CuL_2, (*10*) $CuL_2H_{-1}^{-}$, and (*11*) $CuL_2H_{-2}^{2-}$.

triglycine protonation, pK_{a1} 3.31±0.02 and pK_{a2} 8.09±0.02 (298 K), required for calculations, were obtained from the pH-metric titration curve of the ligand in the absence of the metal against the indicated salt background.

The calculated schemes and equilibrium constants are given in Table 1 together with available published log β values [1–7]. Earlier 11 of the 13 forms we found were detected in [4] by potentiometry (Table 1). We are the first to characterize the complex CuL_2H^{+}. From a comparison of our and published data (Table 1) it is evident that the stability constant of the complex $CuLH^{2+}$ reported in [6] is in error and that earlier determined log β values for $CuLH_{-3}^{2-}$ [2, 3, 6] are widely scattered, probably because of the neglect of the above-mentioned hydrolysis of the ligand.

The calculated parameters of electronic absorption spectra and spin–lattice relaxation of the complexes are given in Table 2. The tabulated values of λ_{max} and ε_{max} for the complexes CuL +, $CuLH_{-1}$, $CuLH_{-2}^{-}$, and $CuL_2H_{-1}^{-}$ are rather close to those reported earlier [1, 2, 5–7]. The spectral parameters in [5] for the compounds $CuLH^{2+}$ (λ_{max} 758 nm and ε_{max} 31 mol^{-1} l cm^{-1}), CuL_2 (λ_{max} 625 nm and ε_{max} 47 mol^{-1} l cm^{-1}), and $CuL_2H_{-2}^{2-}$ (λ_{max} 546 nm and ε_{max} 56 mol^{-1} l cm^{-1}) slightly differ from the respective values in Table 2. The electronic spectra of the remaining complexes in Table 2 were reconstructed in this work for the first time. The molar spin–lattice relaxation coefficients (K_{R1}) of the detected complexes were also determined for the first time (variations in K_{R1} for the compounds $CuL_2H_{-1}^{-}$ and $CuL_2H_{-2}^{2-}$ are considered below).

Comparison of the stability constants of monoligand copper(II) complexes with triglycine (Table 1) and diglycine (GGH) [8], together with the results of molecular mechanics (MM+) calculations (Fig. 3) allows us to deduce certain conclusions as to the structure of the compounds under consideration. The log β value for the complex $CuLH^{2+}$ is higher by 0.48 than that for $CuGGH^{2+}$ [8], probably because of coordination of one more oxygen atom of the second peptide group in addition to the carboxy group and the peptide oxygen atom (Fig. 3). At the same time, the diglycine analog of the complex CuL^{+} is more stable (log β 5.63 [8]), as at similar coordination of di- and tripeptides via the peptide oxygen atom and the amino group (Fig. 3) the basicity of the amino group in diglycine is higher (pK_{a2} 8.24 at 298 K [8]). The diglycine complex $CuGGH_{-1}$ (log β 1.24 [8]) is more stable than the triglycine complex $CuLH_{-1}$ since in the former complex the carboxy group is bound instead of the peptide oxygen atom of the tripeptide (Fig. 3). In the complex $CuLH_{-2}^{-}$, the tripeptide occupies all the four equatorial positions (Fig. 3), and, therefore, the addition of the hydroxide ion to it to form $CuLH_{-3}^{2-}$ should be accompanied by expulsion of the coordinated carboxy group and has an equilibrium constant [log K_{OH} = –18.81 – (–7.06) = –11.75] about

Table 1. Formation constants of copper(II) complexes with triglycine (LH) at 298 K on the background of 1 M KNO_3 in comparison with published data [1–7] (β are equilibrium constants)

No.	Equilibrium	log β	
		this work	published data
1	$Cu^{2+} + LH \rightleftarrows CuLH^{2+}$	1.52±0.06	1.58 [4], 1.49 [5], 2.36 [6]
2	$Cu^{2+} + L^{-} \rightleftarrows CuL^{+}$	5.19±0.03	5.3 [1], 4.80 [2], 5.5 [3], 5.66 [4], 5.30 [5], 5.08 [6], 5.25 [7]
3	$Cu^{2+} + L^{-} \rightleftarrows CuLH_{-1} + H^{+}$	–0.30±0.02	0.1 [1], –0.30 [2], 0.1 [3], –0.13 [4], –0.18 [5], –0.08 [6], –0.16 [7]
4	$Cu^{2+} + L^{-} \rightleftarrows CuLH^{-}_{-2} + 2H^{+}$	–7.06±0.02	–6.9 [1], –7.19 [2], –6.53 [3], –6.8 [4], –6.97 [5], –6.82 [6], –7.02 [7]
5	$Cu^{2+} + L^{-} \rightleftarrows CuLH^{2-}_{-3} + 3H^{+}$	–18.81±0.06	–19.1 [2], –17.4 [3], –18.3 [6]
6	$2Cu^{2+} + L^{-} + LH \rightleftarrows Cu_2L_2H^{3+}$	8.59±0.11	8.8 [4]
7	$2Cu^{2+} + 2L^{-} \rightleftarrows Cu_2L_2^{2+}$	12.18±0.13	13.12 [4]
8	$2Cu^{2+} + 2L^{-} \rightleftarrows Cu_2L_2H_{-2} + 2H^{+}$	1.26±0.08	1.44 [4]
9	$Cu^{2+} + 2LH \rightleftarrows CuL_2H_2^{2+}$	2.30±0.17	1.9 (<2.2) [4]
10	$Cu^{2+} + L^{-} + LH \rightleftarrows CuL_2H^{+}$	6.22±0.05	
11	$Cu^{2+} + 2L^{-} \rightleftarrows CuL_2$	9.18±0.07	10.17 [4], 9.66 [5]
12	$Cu^{2+} + 2L^{-} \rightleftarrows CuL_2H^{-}_{-1} + H^{+}$	3.43±0.05	3.7 [1], 3.20 [2], 3.91 [4], 3.34 [5], 3.23 [7]
13	$Cu^{2+} + 2L^{-} \rightleftarrows CuL_2H^{2-}_{-2} + 2H^{+}$	–4.94±0.03	–4.9 [1], –4.81 [4], –4.62 [5]

2 orders of magnitude lower than that for OH^- binding in the free equatorial position of the dipeptide complex $CuGGH_{-1}$ ($\log K'_{OH}$ –9.52 [8]).

The above structural conclusions are confirmed by spectral data (Table 2). The successive short-wave shift of the absorption maximum in the series $CuLH^{2+}$–CuL^{+}–$CuLH_{-1}$–$CuLH^{-}_{-2}$ reflects the increasing number of coordinated nitrogen atoms (0, 1, 2, and 3, respectively). The largest shift of λ_{max} on transition from $CuLH_{-1}$ to $CuLH^{-}_{-2}$ results not only from coordination of the second deprotonated peptide atom of nitrogen, but also from additional equatorial binding of the carboxy group (Fig. 3). At the same time, the long-wave shift of λ_{max} on transition from $CuLH^{-}_{-2}$ to $CuLH^{2-}_{-3}$ points to replacement of this carboxy group by an OH^- ion which creates a weaker crystal field than the COO^- group and ranks below it in the spectrochemical series [12]. Note that axial coordination of any of these groups would also be accompanied by a long-wave shift of the absorption maximum, but in this case the extinction coefficient (ε_{max}) would increase [13], contrary to what is observed experimentally (Table 2). The higher value of K_{R1} for $CuLH^{2-}_{-3}$ than for $CuLH^{-}_{-2}$ (Table 2) also confirms equatorial coordination of the OH^- group in the complex $CuLH^{2-}_{-3}$ [≡ $Cu(LH_{-2})OH^{2-}$]. In fact, this group should undergo fast proton exchange with water

Table 2. Parameters of electronic absorption spectra and molar spin–lattice relaxation coefficients (K_{R1}) of copper(II) complexes with triglycine (LH) at 298 K (1 M KNO_3)

No.	Complex	λ_{max}, nm	ε_{max}, $mol^{-1}\ l\ cm^{-1}$	K_{R1}, $mol^{-1}\ l\ s^{-1}$
1	$CuLH^{2+}$	790	25.7	1183
2	CuL^{+}	740	39.5	1034
3	$CuLH_{-1}$	660	80.4	469
4	$CuLH^{-}_{-2}$	555	152.1	201
5	$CuLH^{2-}_{-3}$	580	98.4	498
6	$Cu_2L_2H^{3+}$	750	23.3	1014
7	$Cu_2L_2^{2+}$	720	32.5	591
8	$Cu_2L_2H_{-2}$	640	68.4	540
9	$CuL_2H_2^{2+}$	750	42.2	1065
10	CuL_2H^{+}	715	58.4	654
11	CuL_2	660	57.7	514
12	$CuL_2H^{-}_{-1}$	620	68.6	
13	$CuL_2H^{2-}_{-2}$	540	51.3	209

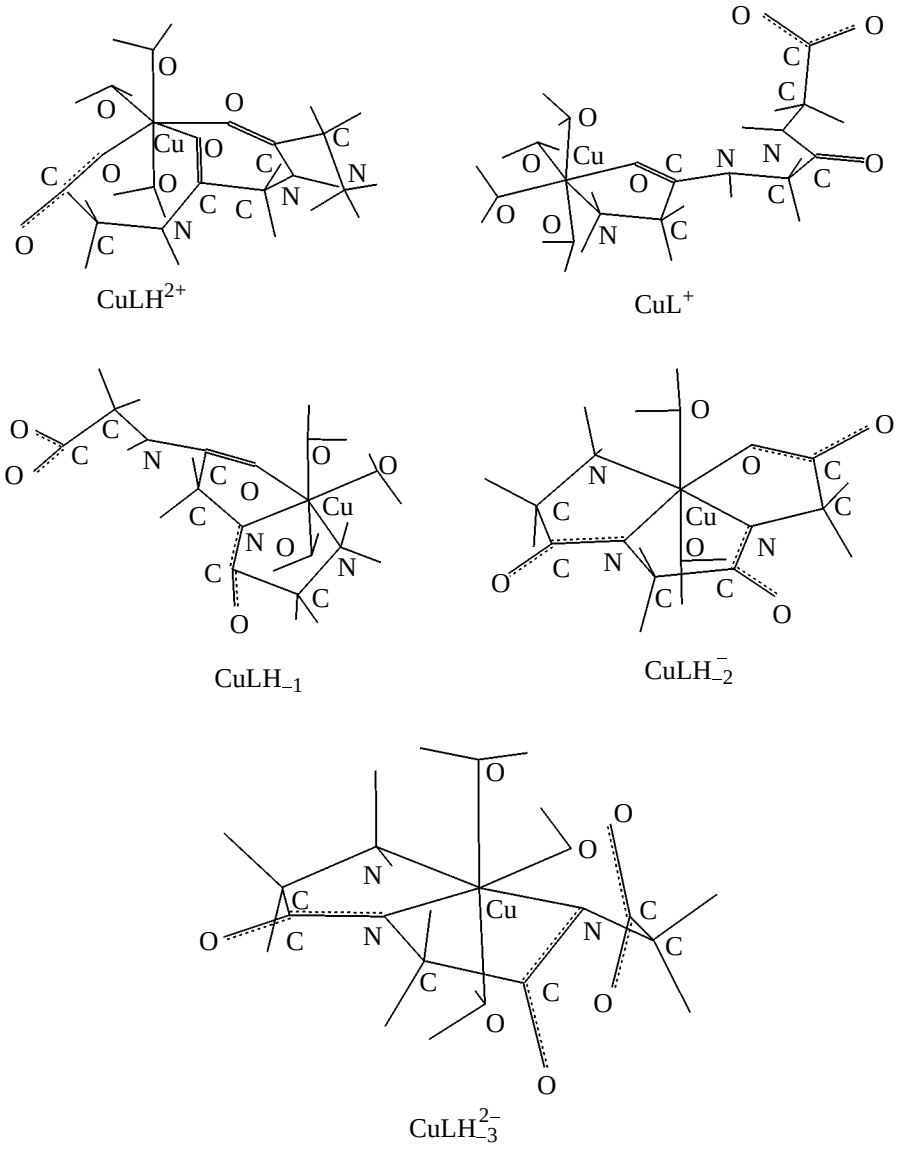


Fig. 3. Structures of monoligand copper(II) complexes with triglycine, calculated by the MM+ method.

protons in bulk solution [14], thus contributing much in the K_{R1} value.

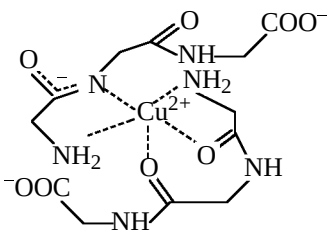
Unlike copper(II)–dipeptide (L'H) systems, where binuclear complexes $Cu_2L'_2H^-_{-3}$ [≡ $Cu_2(L'H_{-1})_2 - OH^-$] with bridging coordination of the hydroxide ion are most frequently formed [8], similar complexes with triglycine are not accumulated because of the competitive binding of the second deprotonated peptide nitrogen atom in the same pH range to give the complex $CuLH^-_{-2}$. On the other hand, binuclear and bis-complexes like $Cu_2L_2H^{3+}$, $Cu_2L_2^{2+}$, $Cu_2L_2H_{-2}$, $Cu_2L_2H_2^{2+}$, $Cu_2L_2H^+$, and CuL_2 (Table 2, nos. 6–11) were not detected in dipeptide systems. Note that the binuclear complexes $Cu_2L_2H^{3+}$, $Cu_2L_2^{2+}$, and $Cu_2L_2H_{-2}$ have no bridging groups which might be common for the coordination spheres of two metal atoms. Otherwise the K_{R1} coefficients of these complexes would be close to zero [15], whereas their actual values are rather high (Table 2). Taking this into account, as well as that the absorption maxima of the binuclear complexes are only slightly shifted to shorter waves compared to those of the constituent mononuclear complexes (Table 2), we can suggest that $Cu_2L_2H^{3+}$, $Cu_2L_2H_2^{2+}$, and $Cu_2L_2H_{-2}$ are formed by addition to the metal of the free carboxy group of the ligand bound to an adjacent metal center. This type of coordination in $Cu_2L_2H^{3+}$ and $Cu_2L_2^{2+}$ resembles that realized in crystalline $CuLCl \cdot 1.5H_2O$, but the structure of the latter comprises infinite metal–peptide chains [16].

Let us pay attention to the close values of the

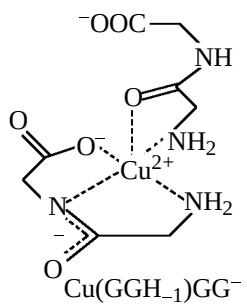
logarithms of the formation constants of the binuclear complexes: $Cu_2L_2H^{3+}$ from $CuLH^{2+}$ and CuL^+ ($\log\beta_6 - \log\beta_1 - \log\beta_2 = 1.88$), $Cu_2L_2^{2+}$ from CuL^+ ($\log\beta_7 - 2\log\beta_2 = 1.80$), and $Cu_2L_2H_{-2}$ from $CuLH_{-1}$ ($\log\beta_8 - 2\log\beta_3 = 1.86$) (the indices at β relate to the numbers of equilibria in Table 1). This fact can be that adjacent metal ions in the compounds $Cu_2L_2^{2+}$ and $Cu_2L_2H_{-2}$ are linked via two free carboxy groups, whereas in the complex $Cu_2L_2H^{3+}$ via only one group (the second group is coordinated in the $CuLH^{2+}$ complex, Fig. 3), but $Cu_2L_2H^{3+}$ is additionally stabilized by H-bond formation between the ammonium group of the zwitter-ionic form of the ligand from the $CuLH^{2+}$ fragment and the second peptide oxygen atom of the anionic form of the ligand from the CuL^+ fragment. Owing to the positive charge on the NH_3^+ group, this bond should be stronger than H bonds N–H···O=C between peptide groups, which stabilize the secondary structure of proteins [17]. In accord with Osterberger and Sjoberg [4], we do not exclude that the binuclear complex $Cu_2L_2H_{-4}^{2-}$ is accumulated in the region of precipitate formation; the disodium salt of this anion has been isolated and characterized by X-ray diffraction [18].

When considering the complexes $CuL_2H_2^{2+}$, CuL_2H^+, and CuL_2, we focused on the differences between the logarithms of the step stability constants of the corresponding mono- and biscomplexes $CuL_2H_2^{2+}$ [$\Delta\log K_9 = \log\beta_1 - (\log\beta_9 - \log\beta_1) = 0.74$], CuL_2H^+ [$\Delta\log K_{10} = \log\beta_2 - (\log\beta_{10} - \log\beta_1) = 0.49$], and CuL_2 [$\Delta\log K_{11} = \log\beta_2 - (\log\beta_1 - \log\beta_2) = 1.20$] (the indices at K relate to the numbers of complexes in Table 2). The resulting $\Delta\log K_{11}$ value of 1.20 is typical of bidentate coordination of ligands [19, 20] and is nicely consistent with the sufficient stability of the five-membered chelate ring formed by L^- (Fig. 3). The $\Delta\log K_9$ value of 0.74 corresponds to monodentate coordination of ligands [19, 20] and is consistent with the low stability of seven-membered chelate rings formed by LH. At the same time, the reduced $\Delta\log K_{10}$ value of 0.49 indicates to additional stabilization of the complex CuL_2H^+, probably due to H-bond formation between the ammonium group of LH and the second peptide oxygen atom of L^-, like in $Cu_2L_2H^{3+}$.

The λ_{max} for the complexes $CuL_2H_{-1}^-$ and $CuL_2H_{-2}^{2-}$ (Table 2) almost coincide with those for diglycine complexes of the same composition [8], implying that one and two ligands, respectively, are coordinated through the amino group and the deprotonated peptide nitrogen atom (in the LH_{-1}^{2-} form). The fact that the step stability constant of the triglycine complex $CuL_2H_{-1}^- \equiv Cu(LH_{-1})L^-$ ($\log K_2 = \log\beta_{12} - \log\beta_3 = 3.73$, Table 1) is higher than that of the similar diglycine complex ($\log K_2$ 3.10 [8]) points to a stronger equatorial chelate coordination of triglycinate L^- as compared to the axial-equatorial coordination of diglycinate GG^- [8].



$Cu(LH_{-1})L^-$



$Cu(GGH_{-1})GG^-$

Note that the formation constant of the complex $CuL_2H_{-2}^{2-} \equiv Cu(LH_{-1})_2^{2-}$ ($\log\beta$ –4.94, Table 1) is much higher that that of the similar diglycine complex $Cu(GGH_{-1})_2^{2-}$ ($\log\beta$ –7.70 [8]). This results from the fact that, unlike the latter complex which has a *trans* structure in crystal [21], the complex $Cu(LH_{-1})_2^{2-}$ has the more stable *cis* structure [22]. According to the reasoning in [22], the preferential accumulation of the *cis* isomer of $Cu(LH_{-1})_2^{2-}$ is associated with the strong σ-donor effect of deprotonated peptide nitrogen atom, which makes *trans* arrangement of two such atoms energetically unfavorable. At the same time, *cis*-$Cu(GGH_{-1})_2^{2-}$ does not exist because of the essential Coulomb repulsion between the carboxy groups of two diglycine ligands in this structure. The complexes $CuL_2H_{-1}^-$ and $CuL_2H_{-2}^{2-}$ display unusual relaxation characteristics which deserve special consideration.

Chemical exchange. The increase in the coefficients of spin–lattice [$(c_M T_{1p})^{-1}$] and spin–spin relaxation [$(c_M T_{2p})^{-1}$] with increasing ligand concentration in the pH range 9–10 (Fig. 4) reflects acceleration of exchange by the deprotonated ligand species L^- between the coordination sphere of the complex $CuL_2H_{-1}^-$ and bulk solution, like in dipeptide complexes with the same composition [8]. Taking account of the calculated accumulation fractions of the complexes $CuL_2H_{-1}^-$, $CuLH_{-2}^-$, and $CuL_2H_{-2}^{2-}$ and their additive contributions to $(c_M T_{2p})^{-1}$, we calculated spin–spin relaxation coefficients for the individual species $CuL_2H_{-1}^-$ (K_{R2}) as a function of ligand L^- concentration (the K_{R2} values for $CuLH_{-2}^-$ and $CuL_2H_{-2}^{2-}$ at pH 9–10 are 410 ± 50 $mol^{-1}\,l\,s^{-1}$). At all the selected pH values (9.0, 9.5, and 10.0, Fig. 4) the K_{R2}–[L^-] dependences are described by Eq. (1) [8].

$$K_{R2} = \frac{P'_a}{\tau_M^a + T_{2M}^a} + \frac{P'_e}{\tau_M^e + T_{2M}^e} = K_a + \frac{P'_e}{(k_1 + k_2[L^-])^{-1} + T_{2M}^e}. \quad (1)$$

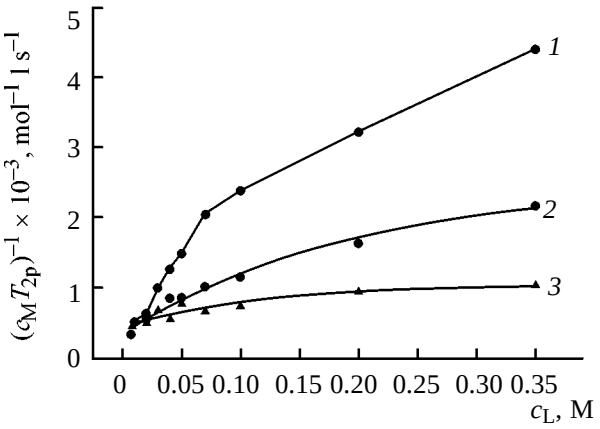


Fig. 4. Molar spin–spin relaxation coefficients $(c_M T_{2p})^{-1}$ vs. ligand concentration (c_L) in the system copper(II)–triglycine ($c_{Cu(II)}$ 5.0×10^{-3} and c_{KNO_3} 1 M, 298 K). pH: (*1*) 9.0, (*2*) 9.5, and (*3*) 10.0.

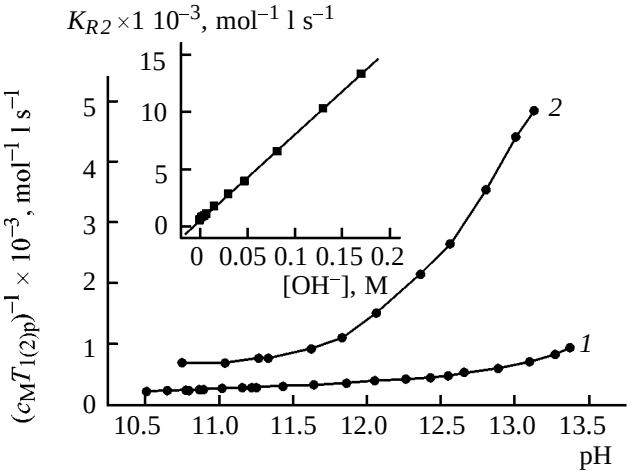


Fig. 5. Parameters (*1*) $(c_M T_{1p})^{-1}$ and (*2*) $(c_M T_{2p})^{-1}$ vs. pH for the system copper(II)–triglycine [the insert shows the calculated molar spin–spin relaxation coefficient of the complex $CuL_2H_{-2}^{2-}$ (K_{R2}) vs. OH^- concentration] ($c_{Cu(II)}$ 4.99×10^{-3}, c_L 0.10, and c_{KNO_3} 1 M, 298 K).

Here indices a and e relate to exchanging protons of axially bound water molecules and an equatorially bound NH_2 group of the ligand L^-, respectively, $P'' = P_M/c_M$, P_M is the mole fraction of bound protons, T_{2M} is their spin–spin relaxation time, τ_M is the lifetime of these protons, and k_1 and k_2 are the first- and second-order rate constants of ligand-exchange reactions, respectively. Calculations from the K_{R2}–[L^-] dependences gave the following results: K_a 400 ± 40 mol^{-1} l 3s^{-1}, k_1 $(6 \pm 3) \times 10^4$ s^{-1}, k_2 $(1.2 \pm 0.1) \times 10^7$ mol^{-1} l s^{-1}, and T_{2M} $(5.6 \pm 0.2) \times 10^{-7}$ s (298 K).

According to [8], k_1 relates to dissociation of the complex $Cu(LH_{-1})L^-$ [reaction (2)].

$$Cu(LH_{-1}) + L^- \underset{k_1}{\overset{k_f}{\rightleftarrows}} Cu(LH_{-1})L^-, K_2. \qquad (2)$$

From the k_1 values and the step stability constants of the complex CuL_2H_{-1} (K_2 $10^{3.73}$, see above) we estimated the rate constant of the forward reaction (2): $k_f = k_1K_2 = 3.2 \times 10^8$ mol^{-1} l s^{-1} (298 K). This k_f value is close to estimates for the rate constants of formation of similar dipeptide complexes (~10^8 mol^{-1} l s^{-1} [8]) and also of copper(II) biscomplexes from monoligand complexes with some anions of α-amino acids {$(2–4) \times 10^8$ mol^{-1} l s^{-1} [23]}. Note that the rate of reaction (2) of the tripeptide complex formation we estimated here for the first time and that it is difficult to determine by any other method.

The exchange rate constant k_2 (1.2×10^7 mol^{-1} l s^{-1}) is close to those found earlier for complexes of the same composition ($CuL_2'H_{-1}^-$) with aliphatic dipeptides [8]. This result is readily explained by the structural similarity of these di- and tripeptide complexes (see above) and to the associative mechanism of ligand substitution in copper(II) complexes [23]. In both types of complexes, blocking of the axial position is a key factor that operates to slow down the attack of the ingoing ligand. Though in tripeptide complexes such blocking is weaker than in dipeptide complexes because of the larger size of the axial–equatorial chelate in $CuL_2H_{-1}^-$, thus facilitating the attack of the outgoing anion L^- in tripeptides is bound stronger than in dipeptides (compare the above-mentioned log K_2 values 3.73 and 3.10); as a result of these opposite tendencies, di- and tripeptide complexes are close in lability.

Let us now consider the complex $CuL_2H_{-2}^{2-}$ which is accumulated in a strongly alkaline medium (Fig. 2). In this case, the spin–lattice and spin–spin relaxation coefficients considerably increase with pH (Fig. 5). For treating the plots in Fig. 5, by the equation –log [OH^-] = (13.89 – pH) we calculated [OH^-] values, using the ionic product of water pK_w of 13.77 (298 K) found in [24] for 1 M solutions of potassium salts (KCl and KBr). Then we calculated the coefficients K_{R1} and K_{R2} for $CuL_2H_{-2}^{2-}$, taking into account the contributions of the complexes $CuL_2H_{-1}^-$ and $CuLH_{-3}^{2-}$ to $c_M T_{1p}$ and $(c_M T_{2p})^{-1}$ as products of their accumulation fractions into the relaxation coefficients of individual species (for example, the K_{R2} values for $CuL_2H_{-1}^-$ and $CuLH_{-3}^{2-}$ were 221 and

537 $mol^{-1}\,l\,s^{-1}$, respectively). Then, using an equation like (1), we calculated the second-order rate constant of proton exchange in the coordination sphere of the complex (k_{OH}) from the dependences of the K_{R1} and K_{R2} coefficients of the complex $CuL_2H_{-2}^{2-}$ on the concentration of OH^-, taking into account the relationship $\tau_M^{-1} = k_{OH}[OH^-]$. As follows from Fig. 5, the K_{R2}–$[OH^-]$ dependence is strictly linear (correlation coefficient r 0.9998), implying the fulfillment of the "slow exchange" condition ($\tau_M >> T_{2M}$). The resulting k_{OH} value $(4.20 \pm 0.02) \times 10^6$ $mol^{-1}\,l\,s^{-1}$ (298 K) nicely fits the K_{R1}–$[OH^-]$ dependence with T_{1M} $(2.5 \pm 0.1) \times 10^{-5}$ s.

The k_{OH} value is not readily interpreted. As shown in [22], the complex $CuL_2H_{-2}^{2-}$ undergoes an unusual reaction (3), where both the forward and reverse processes are catalyzed by hydroxide ions.

$$CuLH_{-2}^{-} + L^- + OH^- \underset{k'_r}{\overset{k'_f}{\rightleftarrows}} CuL_2H_{-2}^{2-} + OH^-. \qquad (3)$$

The rate constants of these processes, determined by the stopped-flow technique, are k''_f 1.26×10^7 $mol^{-2}\,l^2\,s^{-1}$ and k''_r 8.8×10^4 $mol^{-1}\,l\,s^{-1}$ (298 K) [22]. The authors of the latter work, taking into account the *cis* structure of the complex $CuL_2H_{-2}^{2-}$, offered a mechanism of reaction (3), which includes deprotonation of the peptide nitrogen atom of one ligand and protonation of the second atom to form the $Cu(LH_{-2})L^{2-}$ intermediate, in which one of the ligands is connected by hydrogen bond to a free OH^- ion. However, our value of the proton-exchange rate constant k_{OH} is much higher than the value of k''_r. Therefore, the proton exchange cannot be accounted for by the ligand detachment by the reverse reaction (3). Thus we have to accept that the proton exchange results from the momentary coordination of OH^- to $CuL_2H_{-2}^{2-}$, yielding the complex $Cu(LH_{-1})_2OH^{3-}$, followed by dechachment of the OH^- ion whose proton has effectively relaxed in the equatorial position of the metal center. To check this assumption, let us compare our k_{OH} values and the rate constant of the forward reaction of the bis(ethylenediamino)copper(II) cation with the hydroxide ion (k''_f) [13] [reaction (4)]:

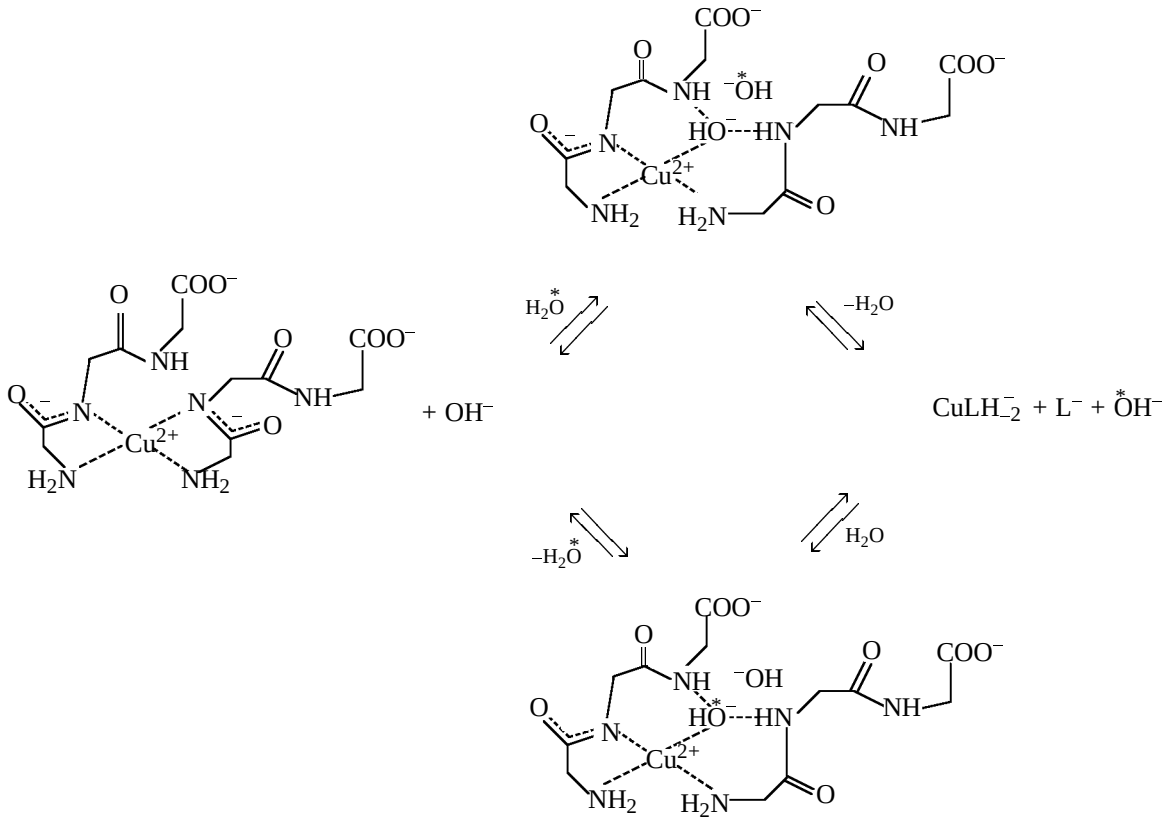
$$Cu(En)_2^{2+} + OH^- \underset{k''_r}{\overset{k''_f}{\rightleftarrows}} Cu(En)_2OH^+, \; K''_{OH}. \qquad (4)$$

From the formation constant of the complex $Cu(En)_2OH^+$ (K''_{OH} 0.41 M) and the rate constant of its dissociation ($k''_r \geq 4.7 \times 10^6$ s^{-1}), reported in [13], we estimated $k''_f = k''_r K''_{OH} \geq 1.9 \times 10^6$ $mol^{-1}\,l\,s^{-1}$ (298 K). Thus, the k_{OH} and k''_r values for similar reactions of two CuN_4 coordination polyhedra are comparable with each other. This fact provides evidence for momentary equatorial coordination of the hydroxide ion to $CuL_2H_{-2}^{2-}$ with expulsion of the amino group of one of LH_{-1}^{2-} ligands to the axial position of the complex $Cu(LH_{-1})_2OH^{3-}$ in the same way as it takes place in $Cu(En)_2OH^+$ [13]. In both cases, the high rates of proton exchange, i.e. short lifetimes of the OH^- ion in the equatorial positions of copper(II), by no means result from fast protonation of the coordinated hydroxide ion by outer-sphere water molecules, but from its competitive expulsion from these positions by the nucleophilic amino groups of chelated ligands, leading to the above-mentioned associative activation of substitution. In fact, in the case of the copper(II) complex with diethylenetriamine $Cu(Dien)OH^+$, when such competition is absent, the rate constant of the proton exchange involving the OH^- ion is as low as 5.0×10^5 s^{-1} (298 K) [14], i.e. it is much lower than the k''_r value (see above). Moreover, the observation of a strictly linear K_{R2}–$[OH^-]$ dependence points to insignificant accumulation fractions of the complex $Cu(LH_{-1})_2OH^{3-}$ under our experimental conditions (Fig. 5).

In view of the above-noted possibility of equatorial coordination of OH^- to $Cu(LH_{-1})_2^{2-}$, the mechanism of reaction (3), studied in [22], should be revised. We propose a new mechanism whose main features are shown in Scheme 1.

In our opinion, the catalytic effect of the OH^- ion in a reverse reaction (3) consists in that it replaces the deprotonated peptide nitrogen atom with simultaneous protonation of the latter by water molecule, which is accompanied by liberation of another hydroxide ion. Then the coordinated OH^- ion deprotonates the second peptide nitrogen atom of an adjacent equatorial LH_{-1}^{2-} ligand to form LH_{-2}^{3-}. As a result, the carboxy group of the latter begins to compete for the equatorial position which is still occupied by the amino group of L^-, thus facilitating detachment of the latter group and completing the reaction. According to the microscopic reversibility principle, a forward reaction (3) occurs in an order reverse to that described above. It starts from attack of the amino group of L^- on the equatorial position of the coordinated carboxy group, followed by replacement of the peptide nitrogen atom of the ligand LH_{-2}^{3-} by the hydroxide ion, protonation of the nitrogen by water molecule, and detachment of the OH^- ion from it. Then the bound hydroxide ion deprotonates an adjacent peptide group L^- whose coordination completes a forward reaction (3). We underline once more the importance of associative activation involving chelating ligands, among them the carboxy group of LH_{-2}^{3-} in the reverse reaction (3) and the amino group of L^- in a forward reaction. Thus, OH^- substitution for the amino group in the complex

Scheme 1.



$Cu(LH_{-1})_2^{2-}$ results in proton exchange, and OH^- substitution for the deprotonated peptide group, in reaction (3).

In general, our present results point to the fact that both the oxygen and the nitrogen atoms of the second peptide group affect essentially both the thermodynamics of formation and the kinetics of chemical exchange reactions of copper(II) tripeptide complexes as compared to dipeptide complexes. The effect of functional groups of more complex tripeptides on the structure, stability, and dynamic behavior of copper(II) complexes deserves further research.

EXPERIMENTAL

The optical densities of the solutions were determined on an SF-46 spectrophotometer with an accuracy of ±1%. The times of spin–lattice (T_1) and spin–spin (T_2) relaxation of water protons were measured on a pulse coherent NMR spectrometer (operating frequency 15 MHz) with an accuracy of ±2 and ±3%, respectively. The paramagnetic relaxation rates ($1/T_{1p}$ or $1/T_{2p}$) were evaluated as differences between measured values of $1/T_1$ or $1/T_2$ in the presence and absence of copper(II). The pH measurements were carried out on a pH-673M instrument with an accuracy of ±0.01 log. unit. Chemical grade copper(II) nitrate and triglycine (chromatographically homogeneous) purchased from Reanal were used. The acidity of the medium was maintained with solutions of analytical grade HNO_3 and KOH, and the salt background was created with chemical grade KNO_3 recrystallized from water.

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