Copper(II) Complexes of Triglycine and Tetraglycine[1,2]

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Abstract: Complex formation of copper(II) ion with triglycine or tetraglycine is specific in its promotion of the ionization of peptide hydrogen. The "aqueous" (D_2O) infrared spectrophotometric measurements show that the frequency of infrared absorption band of peptide carbonyl groups decreases from ~1670 to 1610 cm^{-1} with the decrease of hydrogen ion concentration as the peptide hydrogen is displaced in the course of complex formation. A total of two and three peptide hydrogens are displaced from triglycine and tetraglycine complexes, respectively, and this occurs at biochemically important pH values. Also, the frequency changes of the infrared absorption band of the carboxyl group suggest that this group is involved in metal–ligand coordination at all intermediate hydrogen ion concentrations and is displaced only at very high hydroxyl ion concentrations. Combining the results of infrared spectrophotometric studies and potentiometric measurements, the microscopic natures of the species involved are determined, and the equilibrium constants of successive dissociation steps involving peptide hydrogen ion displacement are computed from potentiometric data at 24.9° and at ionic strength of 0.1 *M* KNO_3.

In the previous study of copper(II)–glycylglycine complexes[3] it was noted that the frequency of the infrared absorption band of the peptide carbonyl group decreases as the proton from peptide nitrogen is displaced through metal–nitrogen coordination. Because more than one peptide linkage is present in triglycine (glycylglycylglycine) and tetraglycine (glycylglycylglycylglycine), and the infrared spectra of these peptides in the absence of metal ion have been investigated previously,[4] it was considered desirable to study the copper(II) complexes of these ligands. The purpose of this research, therefore, is to determine the number of existing species and their structures in solution, which in turn makes possible the measurement of the equilibrium constant for each microscopic step. These findings would be used to compare the properties of the copper(II) complex species formed from triglycine and tetraglycine with the corresponding species of diglycine, as well as with the structures suggested by others for these ligands.

Experimental Section

Methods. The infrared spectral and potentiometric measurements were carried out as described previously.[3]

Materials. Sources of the reagents used are the same as those reported earlier.[3,4]

Calculations. The following reaction steps were found to take place, as will be demonstrated below.

a. Triglycine Complexes

$$Cu^{2+} + L^- \rightleftharpoons CuL^+ \qquad K_1 = \frac{[CuL^+]}{[Cu^{2+}][L^-]} \qquad (1\text{-}1)$$

$$CuL^+ \rightleftharpoons CuA + H^+ \qquad K_{1a} = \frac{[CuA][H^+]}{[CuL^+]} \qquad (1\text{-}2)$$

$$CuA \rightleftharpoons CuB^- + H^+ \qquad K_{1b} = \frac{[CuB^-][H^+]}{[CuA]} \qquad (1\text{-}3)$$

$$CuB^- \rightleftharpoons CuBOH^{2-} + H^+ \qquad K_{1c} = \frac{[CuBOH^{2-}][H^+]}{[CuB^-]} \qquad (1\text{-}4)$$

where $L^- = HA^- = H_2B^- =$ anionic species of triglycine.

b. Tetraglycine Complexes

$$Cu^{2+} + L^- \rightleftharpoons CuL^+ \qquad K_1 = \frac{[CuL^+]}{[Cu^{2+}][L^-]} \qquad (2\text{-}1)$$

$$CuL^+ \rightleftharpoons CuA + H^+ \qquad K_{1a} = \frac{[CuA][H^+]}{[CuL^+]} \qquad (2\text{-}2)$$

$$CuA \rightleftharpoons CuB^- + H^+ \qquad K_{1b} = \frac{[CuB^-][H^+]}{[CuA]} \qquad (2\text{-}3)$$

$$CuB^- \rightleftharpoons CuC^{2-} + H^+ \qquad K_{1c} = \frac{[CuC^{2-}][H^+]}{[CuB^-]} \qquad (2\text{-}4)$$

where $L^- = HA^- = H_2B^- = H_3C^- =$ anionic species of tetraglycine.

For the complexes of triglycine and tetraglycine, graphical methods of Schwarzenbach, *et al.*,[5] were employed to obtain K_1 and K_{1a}, and K_{1a} and K_{1b}, respectively. For example, the relationship between K_{1a} and K_{1b} is as follows, assuming reactions 1-1, 1-2, and 1-3 (or 2-1, 2-2, and 2-3) at $a \sim 2.5$ (a = number of moles of base added per mole of ligand).

$$A_1K_{1b}^2 + B_1K_{1b} + C_1 = 0 \qquad (3)$$

$$A_1 = T_M[H^+]\left\{\frac{(T_LC^2[H^+]}{A} + C\right\} + T_LC[H^+] + A$$

$$B_1 = T_M[H^+]\left\{\frac{2T_LBC[H^+]}{A} + B + CD[H^+]\right\} + T_LB[H^+] + CE + 2DA[H^+]$$

$$C_1 = T_M[H^+]\left\{\frac{T_LB^2[H^+]}{A} + BD[H^+]\right\} + BE + D^2A[H^+]^2$$

$$A = \gamma T_L - \alpha Z$$

$$B = \frac{[H^+]}{K_{1a}}(\alpha - \gamma) + (2\alpha - \gamma)$$

$$C = \frac{3\alpha - \gamma}{[H^+]}$$

$$D = \frac{[H^+]}{K_{1a}} + 1$$

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(2) Abstracted in part from material submitted by M. K. Kim to the faculty of Illinois Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(3) M. K. Kim and A. E. Martell, *Biochemistry*, 3, 1169 (1964).

(4) M. K. Kim and A. E. Martell, *J. Am. Chem. Soc.*, **85**, 3080 (1963).

(5) G. Schwarzenbach, A. Willi, and R. O. Bach, *Helv. Chim. Acta*, **30**, 1303 (1947).

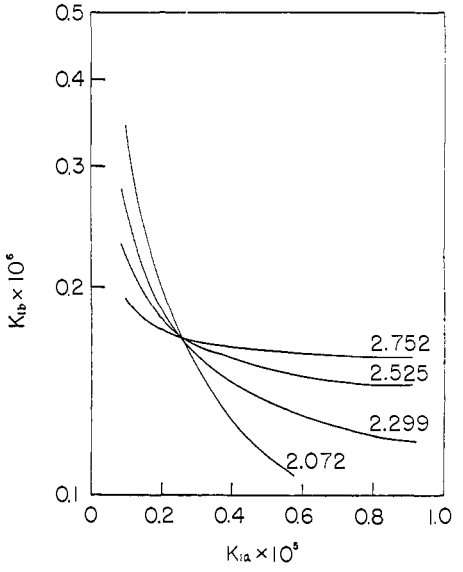


Figure 1. Graphical computation of K_{1a} and K_{1b} for copper(II) complexes of tetraglycine. Numbers on each curve are a values.

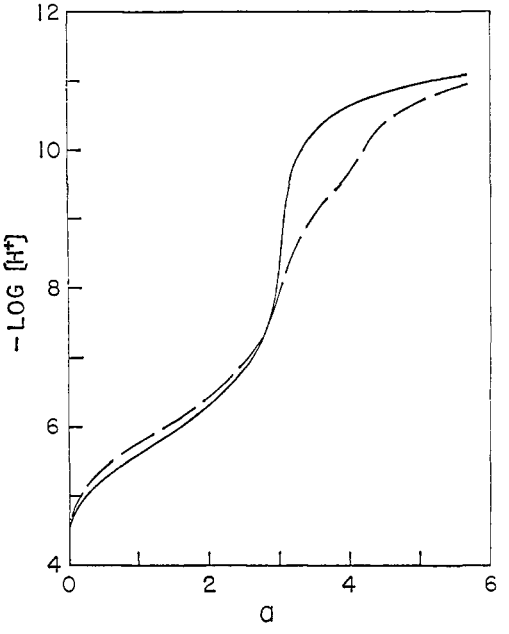


Figure 2. Potentiometric titration of triglycine and tetraglycine in the presence of Cu(II) ion: ———, Cu(II) + GGG, at a = 0, $T_{Cu} = 0.9985 \times 10^{-3}\ M$, $T_{GGG} = 1.000 \times 10^{-3}\ M$; - - - -, Cu(II) + GGGG, at a = 0, $T_{Cu} = 0.9985 \times 10^{-3}\ M$, $T_{GGGG} = 1.006 \times 10^{-3}\ M$ (ionic strength 0.1 M (KNO_3), 24.9°).

$$E = \frac{\alpha}{K_1 K_{1a}}[H^+]^3 + DT_L[H^+]^2$$

$$\alpha = [H^+]^2/K_1^H K_2^H + [H^+]/K_2^H + 1$$

$$\gamma = 1 - [H^+]^2/K_1^H K_2^H$$

$$Z = aT_L + [H^+] - [OH^-]$$

T_L and T_M are total concentrations of ligand and metal species, respectively, and K_n^H refers to the acid dissociation constant of the ligand. At 24.9° and at an ionic strength of 0.1 M KNO_3, the values of pK_1^H and pK_2^H are 3.27 and 7.90 for triglycine and 3.24 and 7.89 for tetraglycine. With known values of K_1 obtained from a similar relationship between K_1 and K_{1a}, the equation (3) is illustrated in Figure 1, where the intersecting point of all curves gives the values of K_{1a} and K_{1b}. The value of K_{1c} of triglycine

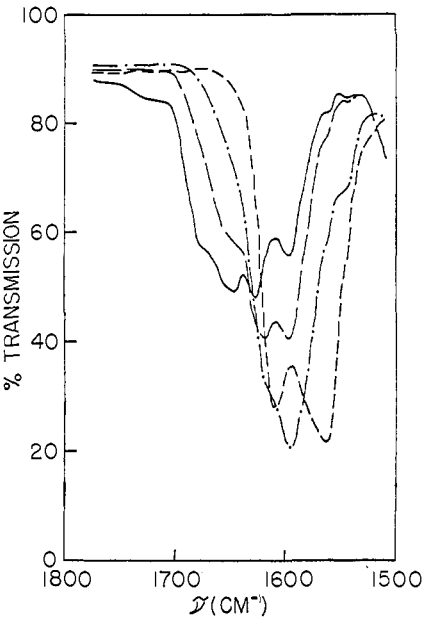


Figure 3. Infrared spectra of copper(II)–triglycine complexes in "aqueous" (D_2O) solutions (1:1); $T_{Cu} = T_{GGG} = 0.250\ M$; ionic strength 1.0, adjusted with KCl: ———, pD 3.95; — —, pD 5.89; -·-·-·, pD 7.82; - - - -, pD ~12.

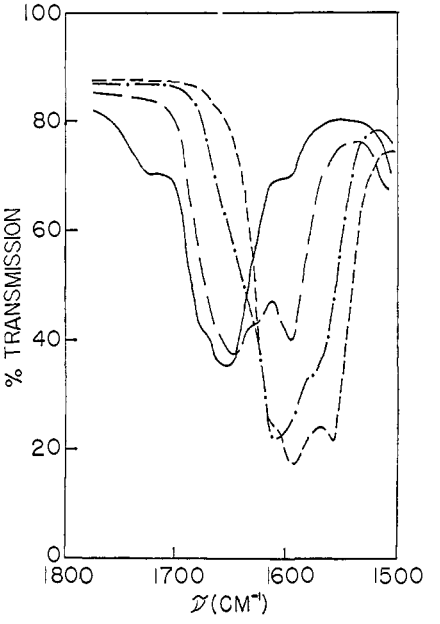


Figure 4. Infrared spectra of copper(II)–tetraglycine complexes in "aqueous" (D_2O) solutions; $T_{Cu} = 0.245\ M$, $T_{GGGG} = 0.241\ M$; ionic strength 1.0, adjusted with KCl: ———, pD 2.80; — —, pD 5.39; -·-·-·, pD 9.35; - - - -, pD 10.52.

complexes was computed by assuming two metal chelate species, CuB^- and $CuBOH^{2-}$ at $a \sim 3.5$. The value of K_{1c} of reaction 2-4 was obtained at $a \sim 3.5$ using the known values of K_1, K_{1a}, and K_{1b}. The calculation of the degree of formation of each species was carried out as described previously.[3]

Results

Potentiometric equilibrium curves for the copper(II) complexes of triglycine and tetraglycine are shown in Figure 2. For solutions having an equimolar ratio (1:1) of metal to ligand, there is a definite inflection at a = 3 in the case of triglycine, whereas a rather slanting curve up to a = 4 is observed for tetraglycine.

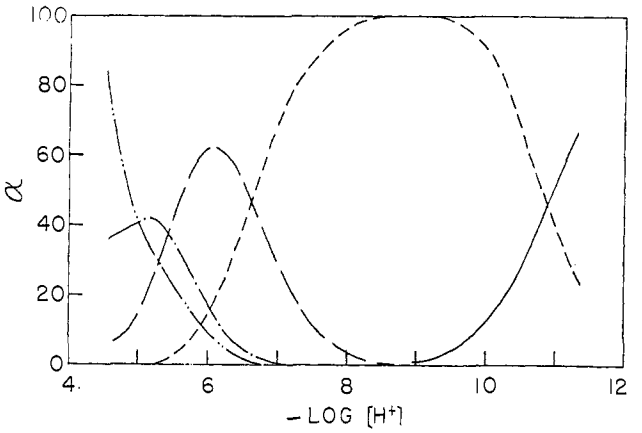


Figure 5. Degree of formation of copper(II)–triglycine complexes in 1:1 metal-to-ligand solution: ···, $\alpha_{Cu^{2+}}$; -·-·-, $\alpha_{CuL^{2+}}$; — —, α_{CuA}; - - - -, α_{CuB^-}; ———, $\alpha_{CuBOH^{2-}}$.

Figures 3 and 4 show the infrared spectra of the 1:1 copper(II)–triglycine and copper(II)–tetraglycine complexes, respectively, in aqueous (D_2O) solutions. In Figure 3, five bands (1720, 1675, 1645, 1630, and 1595 cm^{-1}) are seen at pD ~4. The 1720- and 1675-cm^{-1} bands become weaker as the acidity of the solution is decreased, and at pD 6, there remain only three bands, at 1645, 1620, and 1595 cm^{-1}. As pD increases further, these three bands are replaced by one strong band, at 1595 cm^{-1} with a small shoulder at 1615 cm^{-1}. At very high values of pD, the 1595-cm^{-1} band splits into two parts, with maxima at 1610 and 1563 cm^{-1}.

For the case of copper(II)–tetraglycine complexes, similar features are observed (Figure 4). The spectra at pD 2.80 of Figure 4 are the same as those of the ligand itself in the absence of metal ion. As the pD reaches 5.39, however, bands are apparent at 1645 and 1595 cm^{-1}, and an additional band between them is overlapped by both. At pD 9, all these become one strong band with a maximum at 1610 cm^{-1}, which has a shoulder at 1570 cm^{-1}. At higher pD this shoulder grows larger and finally emerges as a separate band at 1557 cm^{-1}.

The equilibrium constants calculated according to the procedure given in the Experimental Section are listed in Table I. The degree of formation of each species for copper(II)–triglycine complexes in 1:1 solutions is shown in Figure 5 as a function of $-\log [H^+]$.

Table I. Interaction of Cu(II) with Triglycine or Tetraglycine[a]

K_x	Equil. quotients	Log K_x Triglycine	Log K_x Tetraglycine
K_1	$\frac{[CuL^+]}{[Cu^{2+}][L^-]}$	5.5 ± 0.1	5.4 ± 0.1
K_{1a}	$\frac{[CuA][H^+]}{[CuL^+]}$	−5.4 ± 0.1	−5.6 ± 0.1
K_{1b}	$\frac{[CuB^-][H^+]}{[CuA]}$	−6.63 ± 0.02	−6.77 ± 0.02
K_{1c}	$\frac{[CuBOH^{2-}][H^+]}{[CuB^-]}$	−10.9 ± 0.1	
K_{1c}	$\frac{[CuC^{2-}][H^+]}{[CuB^-]}$		−9.0 ± 0.1

[a] 24.9°; μ = 0.1 (KNO_3).

Discussion

The dipolar ionic form of glycine peptides behaves like a monobasic acid. However, when equimolar amounts of Cu(II) ion are added to triglycine, three hydrogen ions are displaced from the 1:1 metal complex in a single step and one more hydrogen ion is displaced at a higher pH value. In the case of the 1:1 copper(II)–tetraglycine solutions, however, a total of four hydrogens are displaced, although the tendency for the displacement to occur is somewhat less than in the case of triglycine. This displacement reaction is highly specific for Cu(II) ions. The only other metal ion reported to undergo this type of reaction is Ni(II), which, however, requires a much higher pH for displacement of peptide protons from polyglycines.

As was discussed in the previous paper[3] for the copper(II)–glycylglycine complexes, a number of studies by other investigators[6-11] supported the concept of coordination of the metal ion to the peptide nitrogen with displacement of peptide protons. Manyak, *et al.*,[12] and Rising, *et al*,[13] were able to crystallize out Cu(II) complexes of triglycine at $a = 2$ and Cu(II) complexes of tetraglycine at $a = 4$, respectively. The crystallographic X-ray studies of copper(II)–triglycine (CuB^- form) by Cooper, *et al.*,[14] also show metal–nitrogen coordination in the solid state.

The infrared absorption spectra of carboxyl and peptide carbonyl groups of the copper(II)–glycylglycine complexes proved to be useful for identifying this type of metal–peptide nitrogen coordination for the metal chelate species in solution.[3] The same is now possible for the copper(II)–triglycine and copper(II)–tetraglycine chelates. Of the five bands at pD 3.95 in Figure 3, with maxima at 1720, 1675, 1645, 1630, and 1595 cm^{-1}, the first three and the last are the same as those for the free ligand in the absence of Cu(II) ion, while the 1630-cm^{-1} band is new and must come from metal complex formation. The main species at this pD is CuL, formula I, where three nitrogens and one carboxyl oxygen are probably weakly coordinated to the metal ion. As the pD is increased, the first three bands of free ligand would be expected to decrease in intensity, and the bands due to complex formation would be expected to increase in intensity. Actually the coordinated peptide carbonyl band at 1620 cm^{-1} and coordinated carboxyl band at 1595 cm^{-1} become very clear at pD 5.89. The complex of importance here is CuA, which has a structure similar to that of CuL, except that the first peptide hydrogen is displaced, as indicated by II.

When both of the two peptide hydrogens are displaced from the complex, the absorption bands of two peptide carbonyl groups in III will shift to lower frequency

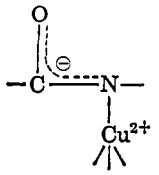
(6) H. Dobbie and W. O. Kermack, *Biochem. J.*, **59**, 246 (1955).
(7) C. B. Murphy and A. E. Martell, *J. Biol. Chem.*, **226**, 37 (1957).
(8) S. P. Datta and B. R. Rabin, *Trans. Faraday Soc.*, **52**, 1123 (1956).
(9) W. L. Koltun and F. R. N. Gurd, *J. Am. Chem. Soc.*, **81**, 301 (1959).
(10) W. L. Koltun, M. Fried, and F. R. N. Gurd, *ibid.*, **82**, 233 (1960).
(11) W. L. Koltun, R. H. Roth, and F. R. N. Gurd, *J. Biol. Chem.*, **238**, 124 (1963).
(12) A. R. Manyak, C. B. Murphy, and A. E. Martell, *Arch. Biochem. Biophys.*, **59**, 273 (1955).
(13) M. M. Rising, F. M. Parker, and D. R. Gaston, *J. Am. Chem. Soc.*, **56**, 1178 (1934).
(14) T. Cooper, H. C. Freeman, G. Robinson, and J. C. Schoone, *Nature*, **194**, 1237 (1962).

Table II. Antisymmetric Carboxyl and Peptide Carbonyl Bands of Copper(II) Complexes of Glycine Peptides as a Function of pD

System	pD	Observed bands, cm^{-1}					
		Peptide carbonyl				Carboxyl	
		−COOH	−C(=O)−NH−	−C(=O)−NH−Cu	−C(=O)−N(⊖)−Cu	$-COO^{\ominus}-Cu$	$-COO^{\ominus}$
Cu-GG[a]	3.58	1720	1670	1625		1598	
	4.25		1670	1625		1598	
	5.18				1610[b]	1610[b]	
	10.65				1610[b]	1610[b]	
Cu-GGG	3.95	1720	1675	1630		1595	
			1645				
	5.89		1645 (w)	1620[b]	1620[b]	1595	
	7.82				1615 (sh)[c]	1595	
	~12				1610		1563
Cu-GGGG	2.80	1720	1675 (w)			1595 (w)	
			1650				
	5.39			1645	1620 (sh)	1595	
	9.35				1610		1570 (sh)
	10.52				1610 (sh)	1592	1557

[a] Kim and Martell.[3] [b] Mergence of two neighboring bands. [c] Shoulder.

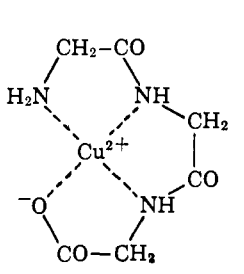
because the resonance that occurs in the following group



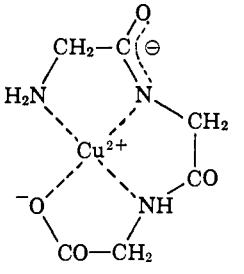
which is formed when a peptide proton is displaced, has considerably lower carbonyl bond order than the corresponding groups shown in I and II. At pD 7.82 the compound CuB^- is the predominant form, the structure of which is represented by III. Since the carboxylate group band is very intense, the 1595-cm^{-1} band (with shoulder) consists of the total effects of two resonance peptide carbonyl bands at 1620 cm^{-1} and one coordinated carboxylate band at 1595 cm^{-1}.

The potentiometric equilibrium curve shown in Figure 2 indicates the displacement of three hydrogen ions, followed by the displacement at higher pH of a fourth hydrogen. Since no more than three hydrogen ions are available from the ligand, and since the hydroxide ion has a stronger affinity for the metal ion than does the carboxylate group, it is concluded that the hydroxide ion will be closer to the metal ion. Thus the carboxylate group will probably occupy one of the more remote coordination positions above and below the plane in which the strong Cu(II) coordination tendencies are directed. Thus the structure of the hydroxoglycylglycylglycinocopper(II) ion is indicated by IV. In view of the structure proposed for IV, it is not surprising that at high pD the strong band observed at pD 7.82 splits into two parts, a resonance peptide carbonyl band at 1610 cm^{-1} and a loosely coordinated (or free) carboxylate band at 1563 cm^{-1}.

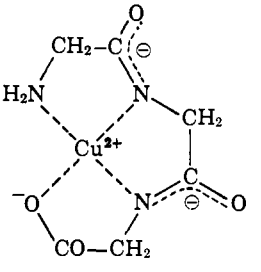
Comparison of the equilibrium constants in Table I with those reported in the literature thus far shows closest agreement with those given by Koltun, *et al.*[11] In order to check the 1:2 metal–ligand complexes which have been suggested previously,[6,11] attempts were made to calculate equilibrium constants corresponding to CuL_2, CuAL, CuA_2, ... with the potentiometric data taken from solutions having 1:2 metal–ligand ratios. No correlation with experimental data was obtained. However, the 1:1 equilibrium constants already obtained from the 1:1 metal–ligand solutions gave quite



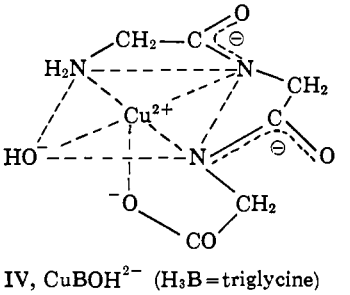
I, CuL^+ (HL = triglycine)



II, CuA (H_2A = triglycine)



III, CuB^- (H_3B = triglycine)



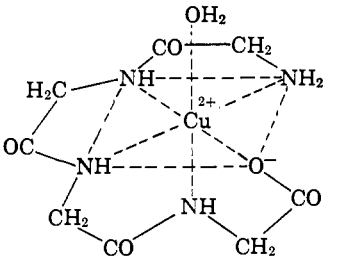
IV, $CuBOH^{2-}$ (H_3B = triglycine)

satisfactory correlation between the calculated equilibrium curve to the experimental equilibrium curve. Therefore it is concluded that no chelates with ligand-to-metal ratios greater than 1:1 are formed in solutions in which the ligand-to-metal ratio is as high as 2:1.

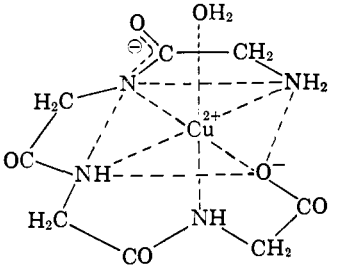
If one takes into consideration that the main difference between tetraglycine and triglycine is that there is one more peptide linkage in the former, the potentiometric equilibrium curve (Figure 2) and infrared spectra (Figure 4) can be readily explained using arguments similar to those given for the triglycine chelates. The spectra at pD 2.80 shown in Figure 4 are just what is expected from the ligand itself, because at this low pD no appreciable amount of metal complex is present. But as the hydrogen ion concentration is decreased, there is an increase in absorption at longer wavelength, as strong coordination with carboxylate and amino groups and weak coordination with peptide carbonyl groups occur. The intermediate structures V, VI, and VII represent steps in chelate formation with increasing pH.

Finally, the mergence of the peptide carbonyl frequencies at 1610 cm^{-1} at pD 9.35 corresponds to the formation of the complex CuC^{2-}, the most probable structure of which is indicated by VIII. Furthermore, it is also interesting to note that the 1570-cm^{-1} band starts to appear at this pD (9.35), in contrast to the behavior of the corresponding triglycine complex, which does not show this high wavelength band until a much higher pD is reached (~12). Thus the carboxylate group in the plane of strong Cu(II) coordination is displaced readily to the more weakly bound positions above or below the plane, since there are three peptide nitrogens and one amino nitrogen to occupy the four planar positions. With triglycine there are only three nitrogens in all, so that the carboxylate group remains strongly bound until very high pH is reached. The carboxylate group is finally displaced at very high pD, where the more strongly bound hydroxide ion becomes available to occupy the fourth planar position.

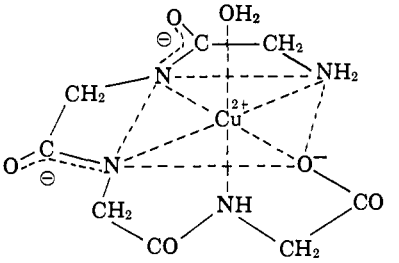
Table II shows the observed infrared absorption bands of carboxyl and peptide carbonyl groups in Cu(II) complexes of glycine peptides. All the band shifts are similar, but the band due to the uncoordinated (or loosely coordinated) carboxyl group at high pD is seen only in the case of triglycine and tetraglycine. These interesting shifts in the infrared frequencies of the carboxylate group and corresponding shift in its coordinate bond strength provide further evidence of strong carboxyl group involvement in metal–ligand coordination for copper(II)–glycylglycine and –triglycine complexes, contrary to what Rabin[15] has suggested for copper(II)–glycylglycine complexes. If carboxyl groups were not to participate in strong coordination to metal ion at all intermediate pH values, and the 1560–1563-cm^{-1} band is merely an absorption of the free carboxyl group, the next nitrogen of which has lost its hydrogen, this band should appear at much lower pD values, that is, as soon as the third hydrogen is displaced from copper(II)–triglycine complex. At the most interesting pH values for biological systems, the CuB^- is found to be the most predominant form (Figure 5). In the case of tetraglycine CuC^{2-}, as well as CuB^-, is important.



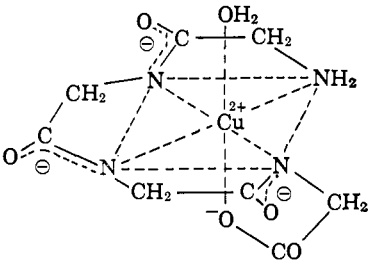
V, CuL^+ (HL = tetraglycine)



VI, CuA (H_2A = tetraglycine)



VII, CuB^- (H_3B = tetraglycine)



VIII, CuC^{2-} (H_4C = tetraglycine)

(15) B. R. Rabin, *Trans. Faraday Soc.*, **52**, 1130 (1956).