that the enol-keto ratio for a given pair of tautomers at equilibrium in solution depends markedly on the polarity of the solvent, and that this ratio tends to be greatest in the least polar solvents (Gould, 1959). Thus it is feasible that an enol-keto shift in the s-RNA or messenger RNA or both may account for a shift in the code for poly-U from phenylalanine to leucine and isoleucine as the alcohol concentration is increased.

ACKNOWLEDGMENTS

We wish to thank Mrs. Fanyela Weinberg for her excellent technical assistance during this investigation and Dr. L. T. Webster, Jr. for his help during the preparation of this manuscript.

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Copper(II) Complexes of Glycylglycine*

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The study of copper(II)-glycylglycine complexes in aqueous solutions by infrared and visible spectrophotometric methods, as well as by potentiometric *p*H measurements, provides evidence for the structures of all the metal complex species present. The frequency changes of the infrared-absorption bands of the carboxyl and peptide carbonyl groups that occur in the course of complex formation gives the first direct proof of the displacement of protons from the peptide nitrogen atoms by reaction with metal ions in solution. The molar absorptivities of each species in the visible spectral region are reported. All the equilibrium constants are calculated for a medium of 1.0 M KCl at 24.9°.

The copper complexes of polypeptides have been studied extensively by many workers mainly because of their biological significance in enzyme reactions. General features of the reactions occurring in aqueous copper-peptide solutions had been worked out by Dobbie and Kermack (1955) from potentiometric and visible-spectrophotometric measurements. By crystallization of certain species (Manyak *et al.*, 1955), or by varying the functional groups of the ligands (Datta and Rabin, 1956), some of the structures of these complexes were inferred. More recently, Koltun and co-workers (Koltun and Gurd, 1959; Koltun *et al.*, 1960, 1963) reported the reactions and species present in solution by potentiometric, kinetic, ultraviolet, and visible-spectral methods. Although all these workers agree that copper-complex formation involves displacement of the peptide hydrogen, there remains disagreement in the number of species present and their structures.

As has been shown for the infrared spectra of glycylglycine (Kim and Martell, 1963), the frequency changes of peptide carbonyl groups at various *p*D values in aqueous solution suggest that it might be possible to find evidence for the binding sites of the corresponding metal complexes by infrared spectrophotometric measurements. The results of such infrared measurements of complexes in solution are of further interest for comparison with the solid-state spectra of the same compounds, the only known example of which was reported by Rosenberg (1957).

Since copper complexes show interesting visible-color changes depending on the number of peptide linkages in the ligand, and on the *p*H of the solution, the visible-spectra and potentiometric measurements are studied simultaneously in the same sample of solution in order to provide further evidence for the identities of all the species formed in solution.

EXPERIMENTAL

Infrared Spectral Measurements.—The method employed is the same as the one described previously (Kim and Martell, 1963) except that exact amounts of metal ion were added to the sample solutions to make the metal-to-ligand ratio 1:1 and 1:2. In both cases, the ligand concentration was −0.20 M. The infrared spectrum of a crystalline sample of aquoglycylglycino-Cu(II) prepared by the method previously described (Manyak *et al.*, 1955) was measured in D_2O as a solvent to check the spectra obtained by the combination of metal ion and ligand in solution.

* This investigation was supported by a research grant (GM 10834-03) from the U. S. Public Health Service, National Institute of General Medical Sciences. 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.

Potentiometric and Visible-Spectral Measurements.—The potentiometric-titration technique has been described in detail (Lenz and Martell, 1964). In order to measure the visible spectra with the same solution as the one used for potentiometric measurements, a potentiometric-titration cell was constructed with an additional inlet and outlet to a specially adapted Beckman Pyrex cell (Beckman no. 75152). The experimental solution was circulated through the cell by a peristaltic pump purchased from the Greiner Scientific Corp., New York. The cell holder and compartment fitted to the Pyrex cell was specially designed to maintain the same temperature as that of the potentiometric-titration cell by circulating water through the compartments of both the absorption and titration cells from the same water bath. Spectra were obtained with a Cary Model 11 recording spectrophotometer. Rectangular Pyrex cells were used in the reference beam. Measurements were made at 24.9° and an ionic strength of 1.0 M regulated by the addition of KCl. Metal-ion concentrations were ~0.020 M and the ratio of metal ion to ligand concentration was 1:1 and 1:2, respectively.

Potentiometric measurements were employed to determine deuterium-ion concentrations for the infrared studies, and for the determination of hydrogen-ion concentrations for the combined spectrophotometric-potentiometric titrations. Hydrogen-ion concentrations were measured as described previously (Lenz and Martell, 1964). For D_2O solutions, the *p*H-meter glass-calomel-electrode system was calibrated for D^+ concentration with solutions of DCl, $DC_2H_3O_2$, and NaOD. The *p*H-meter response was linear with respect to log $[H^+]$ or log $[D^+]$ for H_2O and D_2O solutions under conditions such that the ionic strength did not differ significantly from that of the supporting electrolyte.

Reagents.—The ligand used was the same as that reported previously (Kim and Martell, 1963). Analytical grade copper(II) nitrate and copper(II) chloride were purchased from J. T. Baker Chemical Co., Phillipsburg, N.J. The copper(II) chloride employed in infrared measurements was recrystallized in D_2O twice in a vacuum deisccator. All metal-salt solutions were standarized by titration with EDTA in accordance with the method described by Schwarzenbach (1956).

Calculations.—On the basis of information to be developed, the following reaction steps were found to take place:

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

$$CuL^+ \rightleftharpoons CuA + H^+ \qquad K_{1a} = \frac{[CuA][H^+]}{[CuL^+]} \tag{2}$$

$$CuA \rightleftharpoons CuAOH^- + H^+ \qquad K_{1b} = \frac{[CuAOH^-][H^+]}{[CuA]} \tag{3}$$

$$CuA + CuAOH^- \rightleftharpoons Cu_2A_2OH^- \qquad K_D = \frac{[Cu_2A_2OH^-]}{[CuA][CuAOH^-]} \tag{4}$$

$$CuA + L^- \rightleftharpoons CuAL^- \qquad K_{2a} = \frac{[CuAL^-]}{[CuA][L^-]} \tag{5}$$

$$CuAOH^- \rightleftharpoons CuA(OH)_2^{2-} + H^+ \qquad K_{1c} = \frac{[CuA(OH)_2^{2-}][H^+]}{[CuAOH^-]} \tag{6}$$

where $HL^\pm$ = dipolar ionic species of glycylglycine, $HA^- = L^-$, the anionic species of glycylglycine.

Mass balance relationships of metal and ligand species were combined with the appropriate electroneutrality relationships to give equations for the calculation of equilibrium constants. The following terms are defined: T_M = total concentration of metal species; T_L = total concentration of ligand species; K_1^H and K_2^H = first and second dissociation constants of ligand; a = moles of base added per mole of ligand present; $\alpha = [H^+]^2/K_1^H K_2^H + [H^+]/K_2^H + 1$; $\gamma = -([H^+]^2/K_1^H K_2^H) + 1$.

In a region between $a = 1$ and $a = 2$ for solutions containing 1:1 molar ratio of metal to ligand, reactions (1) and (2) are predominant (curve B, Fig. 1). By combining only these two reactions, the following relationship for K_1 and K_{1a} was derived.

$$K_1 = \frac{[H^+](\alpha Z - \gamma T_L)\{K_{1a}(2\alpha - \gamma) + [H^+](\alpha - \gamma)\}}{K_{1a}\{T_M(2\alpha - \gamma) - (\alpha Z - \gamma T_L)\} + [H^+]\{T_M(\alpha - \gamma) - (\alpha Z - \gamma T_L)\}}$$

$$\cdot \frac{1}{K_{1a}(2T_L - Z) + [H^+](T_L - Z)}$$

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

The values of K_1 and K_{1a} were evaluated graphically as described by Schwarzenbach *et al.* (1947). The same procedure was used to calculate K_{1b} and K_D for reactions (3) and (4), where $[Cu^{2+}]$ and $[L^-]$ were neglected. Calculation of K_{1a} was from data taken at very high *p*H (>11), with the aid of the previously determined values of K_{1b} and K_D. Calculation of K_{2a} was made for solutions having a 1:2 molar ratio of metal to ligand by assuming that all reaction steps from (1) to (5) are represented, and by using the calculated values of K_1, K_{1a}, K_{1b}, and K_D. ($[Cu^{2+}]$ was neglected as negligibly small since the ligand was present in excess.)

The concentrations of each species obtained with all these known constants at all *p*H values were employed to compute the molar absorptivities of each species over the full wavelength range of the visible spectrum. Absorbance A at a certain wavelength and a certain *p*H value is expressed as:

$$A = b(\epsilon_{Cu^{2+}}[Cu^{2+}] + \epsilon_{CuL^+}[CuL^+] + \epsilon_{CuA}[CuA] + \epsilon_{CuAOH^-}[CuAOH^-] + \epsilon_{Cu_2A_2OH^-}[Cu_2A_2OH^-] + \epsilon_{CuAL^-}[CuAL^-])$$

where b = cell length and ϵ = molar absorptivity. In the region where all species except two (or three) are negligible, the molar absorptivities of those two (or three) species can be obtained by solving simultaneous linear algebraic equations with two (or three) unknowns.

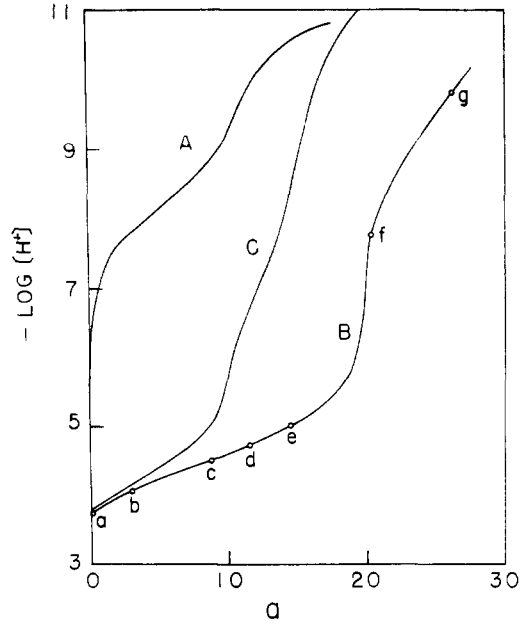


FIG. 1.—Potentiometric titration of glycylglycine (GG) in the absence and in the presence of Cu(II) ion. Curve A, GG; curve B, Cu(II) + GG; curve C, Cu(II) + 2 GG. At $a = 0$, $T_{Cu} = 0.02050$ M, $T_{GG} = 0.02050$ M (for curve B); $T_{GG} = 0.04100$ M (for curve C).

All the above calculations were computed with the aid of an IBM 1620 computer at the Computation Center of Illinois Institute of Technology, and a few points were selected to be checked by hand calculations.

RESULTS

Potentiometric-equilibrium curves for solutions containing 1:1 and 1:2 molar ratios of metal ion to ligand are given in Figure 1, together with one for ligand in the absence of metal ion. The 1:1 solution shows a strong inflection at $a = 2$ and a weak inflection at pH ~ 9. On the other hand, an inflection occurs at $a = 1$ for 1:2 solutions and further weak inflections occur at $a = 1.5$ and $a = 2$ (curve C, Fig. 1).

Examples of the visible spectra obtained for 1:1 solutions are shown in Figure 2. The designations on the spectral curves correspond to the points on the titration curve. The spectra "a," obtained before base is added, mainly come from free metal ion and the first metal complex, CuL^+. As more base is added to the solution the absorption maximum shifts to lower wavelength and finally the absorbance reaches the highest value at ~650 mμ and pH ~8. Above this pH the absorption maxima do not move to other wavelength regions but absorbance is decreased slightly, which is owing partly to the volume increase of the solution on the addition of base, and partly to the slightly lower absorptivities of the alkaline forms of the metal chelate.[1] The spectra obtained for 1:2 solutions were similar except for the fact that the absorption maxima at high pH values occur at ~630 mμ.

Infrared spectra of solutions having 1:1 molar ratios are shown in Figure 3. At relatively high deuterium-ion concentrations, pD (i.e., $-\log [D^+]$) about 3, four absorption bands are found in the carbonyl region; 1720 cm^{-1}, 1670 cm^{-1}, 1625 cm^{-1}, and 1598 cm^{-1}. As pD is increased, the 1720 cm^{-1} band disappears entirely and the remaining three bands merge together to give one

[1] Fig. 2 does not show an isosbestic point because of volume increase of the solution.

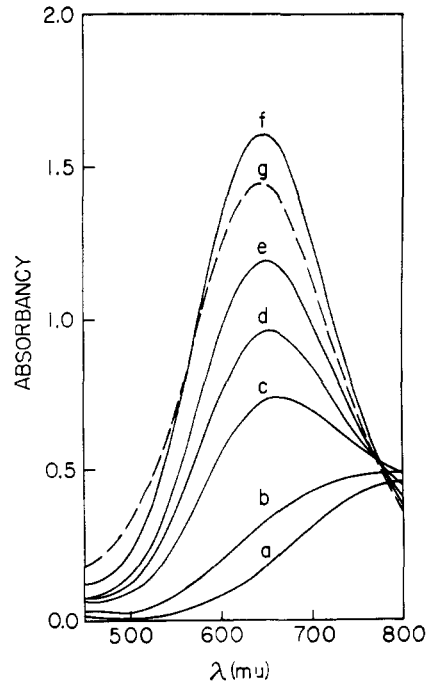


FIG. 2.—Visible spectra of Cu(II)-glycylglycine complexes in aqueous solutions: (a) pH = 3.75; (b) pH = 4.07, (c) pH = 4.52, (d) pH = 4.75, (e) pH = 5.00, (f) pH = 7.76, (g) pH = 9.83.

TABLE I
INTERACTION OF Cu(II) WITH GLYCYLGLYCINE[a]

Equilibrium Quotient, K_X[b]	log K_X
$K_1 = \frac{[CuL^+]}{[Cu^{2+}][L^-]}$	5.42 ± 0.02
$K_{1a} = \frac{[CuA][H^+]}{[CuL^+]}$	−4.38 ± 0.02
$K_{1b} = \frac{[CuAOH^-][H^+]}{[CuA]}$	−9.52 ± 0.02
$K_D = \frac{[Cu_2A_2OH^-]}{[CuA][CuAOH^-]}$	−2.07 ± 0.02
$K_{2a} = \frac{[CuAL^-]}{[CuA][L^-]}$	2.92 ± 0.02
$K_{1c} = \frac{[CuA(OH)_2^{2-}][H^+]}{[CuAOH^-]}$	−12.8 ± 0.1

[a] $t = 24.9°$; $\mu = 1.0$ (KCl). [b] Where HL = H_2A.

band at 1610 cm^{-1}. This occurs at pD about 5.5 and the band persists as pD is further increased. The infrared spectra for 1:2 solutions given in Figure 4 show four bands, 1720 cm^{-1}, 1670 cm^{-1}, 1625 cm^{-1}, and 1596 cm^{-1} in the pD range of 4 ~ 5. At pD 5 ~ 6 the first and one of the last two bands disappear, and two bands remain, 1670 cm^{-1} and ~1600 cm^{-1}. From pD ~7 to ~9, the 1670 cm^{-1} band shifts to lower frequency, giving rise to a shoulder at 1640 cm^{-1}, adjacent to the strong band at 1605 cm^{-1}. At very high pD (> ~12) the shoulder becomes very weak and a broad band with minimum transmission at 1590 cm^{-1} appears.

The equilibrium constants calculated as described in the experimental part are listed in Table I. If the equilibrium constants reported in Table I are compared with those of other workers, they are found to correspond most closely to the values given by Koltun *et al.* (1963). The differences are not more than what would be expected on the basis of the differences in ionic strength. The degree of formation α[2] is obtained

[2] α_c is defined as $\alpha_X = [ML_X]/T_M$.

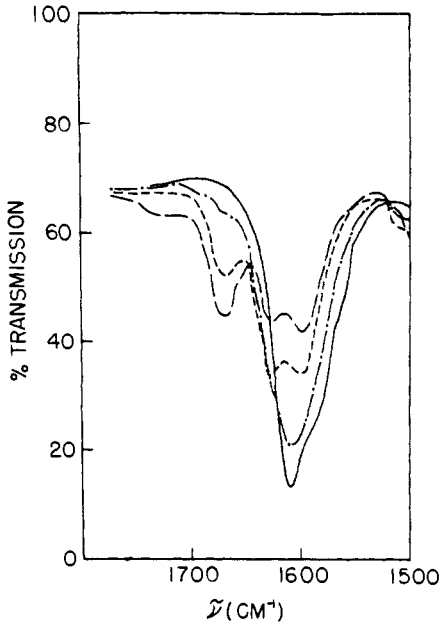


FIG. 3.—Infrared spectra of Cu(II)-glycylglycine complexes in aqueous (D_2O) solutions (1:1). $T_{Cu} = T_{GG} =$ 0.2333 M, and ionic strength 1.0, adjusted with KCl: — — —, pD = 3.58; - - - -, pD = 4.24; —·—·—, pD = 5.18; ———, pD = 10.65.

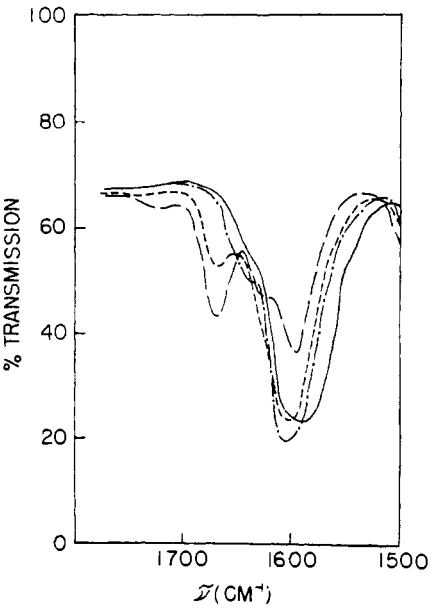


FIG. 4.—Infrared spectra of Cu(II)-glycylglycine complexes in aqueous (D_2O) solutions (1:2). $2T_{Cu} = T_{GG} =$ 0.2333 M, and ionic strength 1.0, adjusted with KCl: — — —, pD = 3.85; - - - -, pD = 5.35; —·—·—, pD = 9.14; ———, pD = 11.82.

TABLE II
ABSORPTION CHARACTERISTICS OF Cu(II)-GLYCYLGLYCINE COMPLEXES[a]

	λ_{max} (mμ)	ε (liter mole^{-1} cm^{-1})[b]
CuL^+	~780	~46
CuA	645	90
$CuAOH^-$	640	78
$Cu_2A_2OH^-$	630	175
$CuAL^-$	615	84

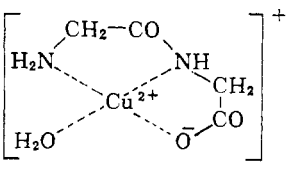
[a] Glycylglycine = HL = H_2A. [b] The errors in the molar absorptivities of all species except $Cu_2A_2OH^-$ are ±5, for $Cu_2A_2OH^-$, ±20.

with the calculated concentration of each species at each pH. The results are given in Figure 5. Figure 6 shows the molar absorptivities of the metal complexes in the visible-spectral region. The absorption maxima and their molar absorptivities are given in Table II. The values given in Table II for CuA, $CuAOH^-$, and $CuAL^-$ are generally in good agreement with the reported values (Dobbie and Kermack, 1955; Koltun *et al.*, 1963). However, the absorption maximum of CuL^+ was found to be higher than the values 720 mμ, ε = 36, reported by Dobbie and Kermack (1955), and higher than the values 735 mμ, ε = 65, reported by Koltun *et al.* (1963). The correct maximum seems to be in the range 775 ~ 800 mμ. The absorption characteristics of $Cu_2A_2OH^-$ have not been reported previously.

DISCUSSION

For solutions having a 1:1 molar ratio of metal ion to ligand, a strong inflection occurs at $a = 2$ for copper (II), while inflections occur at "a" values of 1 for most other metal ions. This had already been noticed by early workers (Dobbie and Kermack, 1955; Manyak *et al.*, 1955; Datta and Rabin, 1956; Murphy and Martell, 1957; Koltun and Gurd, 1959; Koltun, *et al.*, 1960, 1963). Dobbie and Kermack (1955) suggested two possible explanations: (1) The first complex formed, CuL^+, coördinates with a hydroxyl ion; or (2) a proton is dissociated from the ligand. They favored the former interpretation, in accordance with their visible-spectral data. Later Manyak *et al.* (1955) crystallized the compound corresponding to CuA from aqueous solution and suggested the displacement of proton from the peptide linkage. Datta and Rabin (1956) supported this explanation since the second inflection was not observed when a methyl group was substituted for the peptide hydrogen of glycylglycine. Recently Koltun and co-workers reached the same conclusion from their studies of the catalytic hydrolysis of *p*-nitrophenyl acetate by this compound.

The aqueous infrared spectra obtained in this study give the first direct evidence for the displacement of the proton from the peptide nitrogen atom by the metal ion. The four absorption bands that appear in the carbonyl region at low pD values are un-ionized carboxyl (1720 cm^{-1}, weak), peptide carbonyl with an adjacent positive ammonium group (1670 cm^{-1}), peptide carbonyl with neutral α-nitrogen atom (1625 cm^{-1}), and ionized carboxyl (1598 cm^{-1}). At the corresponding pD for the free ligand (Kim and Martell, 1963), however, only the first two bands are observed in the same spectral region. It is seen therefore that at this pD some of the peptide has combined with the metal ion, with displacement of protons from the terminal amino and carboxyl groups. The following structure is therefore suggested for the metal chelate, CuL^+:



I

In solutions of higher pD, the un-ionized carboxyl band disappears completely and the other three bands shift to give one new band at 1610 cm^{-1}. This 1610

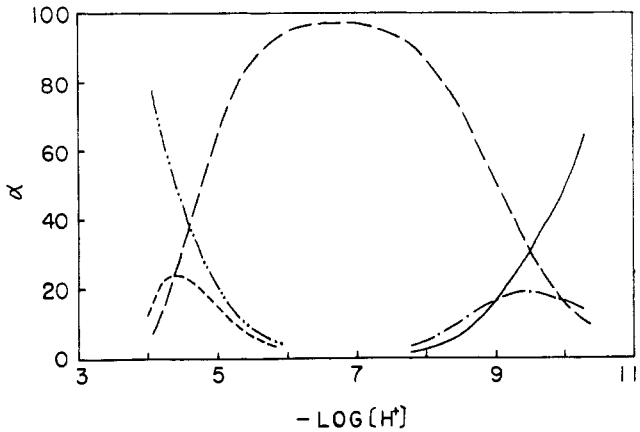
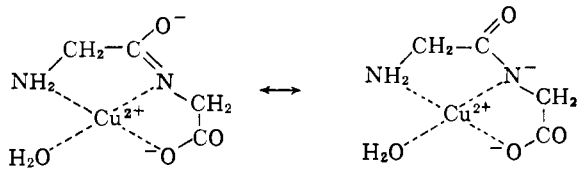


FIG. 5.—Degree of formation of Cu(II)-glycylglycine complexes in 1:1 metal to ligand solution: ··—··—, $\alpha_{Cu^{2+}}$; - - - -, $\alpha_{CuL^{+}}$; — — —, α_{CuA}; ———, $\alpha_{CuAOH^{-}}$; —·—·—, $\alpha_{Cu_2A_2OH^{-}}$.

cm^{-1} band appears at pD $\sim$ 5.5 and maintains its frequency at higher pD values. This is consistent with the potentiometric titration curve, where the neutralization of two protons is shown to occur as a single step for a 1:1 molar ratio of ligand to copper(II) ion. In view of these observations, the following resonance structures are suggested for CuA.



II

Since the bond order of the peptide carbonyl linkage is reduced by coordination with the metal ion and simultaneous displacement of a proton, the infrared peptide carbonyl band would shift to lower frequency. Rosenberg (1957) has observed two bands in this region from the infrared spectra of the deuterated product of crystalline CuA (monohydrate). He assigned the 1613 cm^{-1} band to the ionized carboxyl group and the 1541 cm^{-1} band was considered an amide I band shifted from its original position of 1678 and 1657 cm^{-1} for the ligand, as a result of participation of the peptide linkage in coordination of the metal ion. The aqueous solution spectra, on the other hand, do not show an absorption band near 1541 cm^{-1}. Also the deuterated form of crystalline CuA monohydrate prepared in this investigation does not have an absorption band near 1541 cm^{-1} (measured with the KBr pellet technique). Since the 1610 cm^{-1} band was observed, it appears that the 1541 cm^{-1} band reported by Rosenberg must have been caused by incomplete deuteration of the crystalline chelate.

The titration curve shows another weak inflection at high pH but aqueous infrared spectra show no further frequency changes in the absorption maxima at this pH. Therefore it can be concluded that the binding site of the metal ion at this pH is not significantly different from that which is present at pH 6. The displacement of hydrogen ion from a coordinated water molecule has been predicted (Dobbie and Kermack, 1955; Datta and Rabin, 1956; Koltun and Gurd, 1959). No frequency changes are expected as a result of loss of a proton from the complex $CuA(H_2O)_n$ to give $Cu(OH)A(H_2O)^{-}_{n-1}$. On the basis of the infrared-absorption spectrum, the structure of aqueous $CuAOH^{-}$ can therefore be:

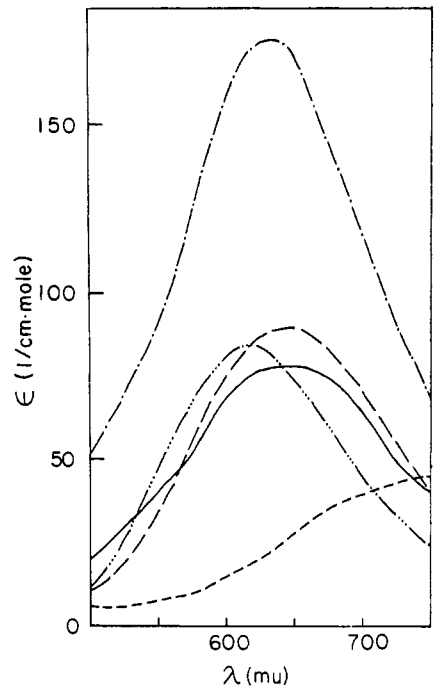
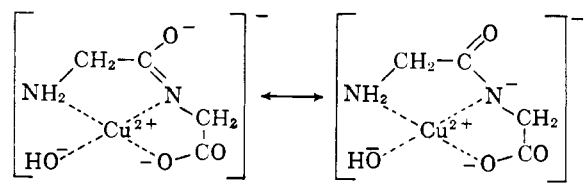


FIG. 6.—Molar absorptivities of each species in the visible region. - - - -, $\epsilon_{CuL^{+}}$; — — —, ϵ_{CuA}; ———, $\epsilon_{CuAOH^{-}}$; —·—·—, $\epsilon_{Cu_2A_2OH^{-}}$; ···—···—, $\epsilon_{CuAL^{-}}$.

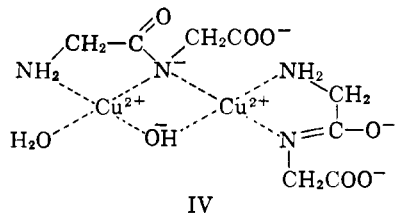


III

This evidence for the nature of the second step in the titration curve also gives further confirmation for the structure of the metal chelate formed in the first step, as indicated by structure II.

If one assumes the formation of species I, II, and III, the titration curve calculated from the equilibrium constants obtained do not fit the experimental data. Datta and Rabin (1956) had similarly found that the equilibrium constant K_{1b} was not constant, and predicted that additional equilibria probably occur, but did not suggest a mechanism. Recently Koltun *et al.* (1960) postulated the species $(CuA)_2OH^{-}$ to obtain a satisfactory curve fit.

Simple dimerization of CuA or CuAOH was considered as a possibility for the additional equilibrium step, but a constant value for the equilibrium constant was not obtained. On the other hand, good values of K_{1b} and K_D are obtained when it is assumed that $Cu_2A_2OH^{-}$ is formed. Since dimeric species of copper(II) complexes in solution with one OH^{-} bridge, as suggested by Koltun *et al.* (1960), are not generally known, the following structure is offered as having a more probable arrangement of coordinate bonds:



IV

At very high pH, the species $CuA(OH)_2^{2-}$ is believed to be formed by combination of a second hydroxyl group with the metal complex and displacement of the carboxyl group from the coordination sphere. This conclusion is supported by the observation of a small shoulder (Fig. 3) at ~1585 cm^{-1} in the aqueous infrared spectra, attributable to a free carboxyl group, which appears when the pD of the solution is increased to 10.65.

Since several equilibrium steps occur in the reactions between Cu(II) and glycylglycine, it was of interest to determine the distribution of each complex species as a function of pH. As is seen in Figure 5, at intermediate pH values which are the most important from the point of view of biochemical interactions, CuA is found to be the most predominant form.

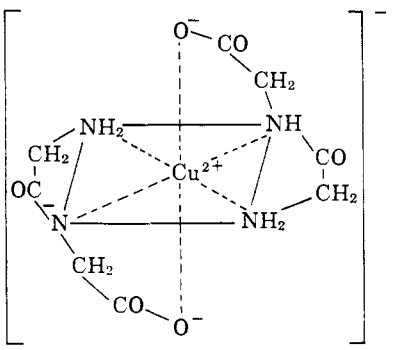
From curve C in Figure 1 it was assumed that the following two reactions occur for 1:2 solutions.

$$CuA + L^- \rightleftharpoons CuAL^- \quad (5)$$

$$CuAL^- \rightleftharpoons CuA_2^{2-} + H^+ \quad (7)$$

and calculation of the corresponding equilibrium constants were attempted. The equilibrium constant corresponding to reaction (7), however, could not be obtained from the data. Only the assumption of reaction (5) gives a constant value of K_{2a} at each experimental point. Titration curves with inflections at "a" values of 2 can therefore be explained as a combination of reactions (1), (2), (3), (4), and (5). This conclusion is consistent with the postulate of Koltun *et al.* (1960) rather than with the assumption of reactions (5) and (7) by Dobbie and Kermack (1955) and by Datta and Robin (1956). Infrared spectra of the aqueous solutions under similar conditions also support this conclusion, since the peptide carbonyl band (1640 cm^{-1}) persists to a high pD, ~9. The degree of formation of species present at 2:1 molar ratio of ligand to metal ion shows that CuA is the main species up to a pH ~ 6.5, but [$CuAL^-$] starts to increase at this pH and finally becomes predominant around pH ~ 9. When the pH becomes higher than 10, the species present in highest concentration is $CuAOH^-$ and very little complex containing more than one ligand per metal ion is present. These conclusions are based on the aqueous infrared spectra, since the 1670 cm^{-1} and 1600 cm^{-1} bands observed at pD = 5.35 are due to CuA and free ligand $HL^{\pm}$, while the 1605 cm^{-1} band with 1640 cm^{-1} shoulder appears as a result of the presence of $CuAL^-$, CuA, $CuAOH^-$, and the free anionic form of the ligand, L^-. The final broad infrared band at high pD would be due to the sum of the absorption bands of CuAL, $CuAOH^-$, and L^-. Since the absorption maximum of CuAOH is 1610 cm^{-1}, as is seen in Figure 3, and those of L^- occur at 1632 cm^{-1} and 1595 cm^{-1} (Kim and Martell, 1963), the broad band observed is in accord with this interpretation.

It is also seen that the absorption maximum of the spectrum at pD = 11.82 for the 2:1 chelate is ~1585 cm^{-1}, compared with that of the spectrum of the 1:1 chelate (Fig. 3) at the corresponding pD (1610 cm^{-1}) and that of the ligand only (1595 cm^{-1}). The infrared frequencies of the 2:1 chelate indicate that the carboxyl groups of the ligands in $CuAL^-$ are not closely coordinated to the metal ion, but merely form two long bonds above and below the plane composed of the metal ion and coordinated nitrogen atoms. Thus the structure of $CuAL^-$ can be represented by V:



V

It is not likely that one ligand occupies three coordination positions of metal ion through its two nitrogen and one carboxyl oxygen atoms and the second ligand supplies only the remaining one position with its amine nitrogen, as suggested by Koltun *et al.* (1960), since the copper(II) ion generally has higher affinity for nitrogen than for oxygen (Martell, 1961). The same argument can be made in relation to the suggestion of Rabin (1956) that the peptide oxygen is involved in coordination instead of the adjacent nitrogen atom.

The significant change in the absorption maximum of the visible spectra of CuA compared to that of CuL^+ is reasonable because in the former a proton is displaced from the peptide nitrogen. On the other hand, absorption maxima for CuA and $CuAOH^-$ are not expected to differ significantly from each other. Since coordination sites of the metal ion in structure IV for $Cu_2A_2OH^-$ are similar to those of $CuAOH^-$, absorption maxima of these two are expected to be quite similar, as observed, while the extinction coefficients of the former would be roughly twice as large as those of the latter. Since the two ligands in $CuAL^-$ form a somewhat stronger field around the metal ion than is true of the 1:1 complexes, the absorption maximum is seen to occur at a somewhat lower wavelength.

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