between the π^* and t_{2g} orbitals increases. Energy mismatch would lead to less effective interaction between the two orbitals, thus a smaller lowering of t_{2g} in the complex and a smaller degree of π bonding between the t_{2g} metal orbitals and the π^* orbital.

From Figure 2 we see that the Cusachs method indicates that both empty π orbitals in DMCA lie higher in energy than the empty π orbitals of acetonitrile. Interaction between either of the DMCA π orbitals and the metal t_{2g} orbitals then would lead to the prediction of a decreased value for *Dq* in the DMCA complex of nickel because of the increased energy mismatch. The π_z^{**} orbital is raised the largest amount (1.11 eV) while the π_y^* orbital lies 0.40 eV higher in energy.

The coefficient of the p_π atomic orbital on the nitrile nitrogen in the π_y^* orbital is larger than that of the corresponding atomic orbital in the $p_z\pi^{**}$ molecular orbital of DMCA. The coefficients are 0.9205 and 0.8451, respectively. The Cusachs calculations on acetonitrile give 0.9011 for the coefficient of the nitrogen p_π atomic orbitals in the empty π^* orbitals. From the Cusachs calculations then, a poorer energy match for both empty π orbitals of DMCA and the metal t_{2g} orbitals would contribute to a lower *Dq* for the DMCA complex than for the acetonitrile complex. In addition, the overlap between the π_z^{**} molecular orbital in DMCA and the metal t_{2g} orbitals should be smaller than the overlap between an empty π^* orbital in acetonitrile and the t_{2g} orbitals. This also should lead to a lower *Dq* for CH_3CN. The better overlap between the π_y^* molecular orbital of DMCA and the t_{2g} metal orbitals compared to the overlap between an acetonitrile π orbital and the t_{2g} metal orbitals is not enough to overcome the other three factors.

Prediction of the relative degree of metal–ligand π bonding from the WH calculations is essentially the same. The results of either calculational method then can be used qualitatively to explain the lower *Dq* value for the DMCA complex and the better donor strength of DMCA compared to CH_3CN.[13]

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Thermodynamics of Ion Association. XVII. Copper Complexes of Diglycine and Triglycine

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Abstract: A sensitive differential calorimeter has been used to measure the enthalpy changes at 25° associated with the formation of copper diglycine and copper triglycine complexes and with the dissociations of the peptide hydrogen atoms at higher pH. The data have been combined with potentiometric measurements to yield ΔG, ΔH, and ΔS values at an ionic strength of 0.10 *M* for the various equilibria involved. At low pH, the values of the thermodynamic functions favor a bidentate structure for the species CuL^+ similar to that in the solid state, in which the copper is bonded to the terminal $-NH_2$ and the oxygen of the first peptide group. At higher pH, the thermodynamic data are discussed in terms of the important factors involved in the labilization of the peptide hydrogen atoms when the ligands are bound to the copper ion.

In recent years there has been considerable interest in the elucidation of the structures of the complex species formed in aqueous solutions of metal peptides because of their biological significance in enzyme reactions. The copper complexes of the polyglycines are of particular importance since dissociation of the peptide hydrogen atoms is facilitated in reactions which take place in the physiological range of pH. The structures of the complexes formed have been characterized in the solid state by X-ray methods.[2,3] In solution, however, there has been considerable disagreement as to the nature of the ligand atoms involved in coordination with the copper ion. In the absence of any unequivocal method for the determination of the structures in solution, they have generally been inferred from free-energy, kinetic, and spectral measurements.[4–10] There is now strong evidence, both from infrared absorption studies and from the observation that the peptide hydrogen atoms in some of the metal complexes readily undergo ionization, that the peptide group is involved

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in the coordination with the metal ion. After the proton elimination, there is little doubt that the nitrogen atom of the peptide group is bound to the copper atom. In the cationic complexes, CuL^+, however, both oxygen and nitrogen peptide coordinations in solution have been proposed by different workers; interaction with either atom might be expected to enhance the acidity of the peptide hydrogen. A linear relationship between log (association constant) for a number of copper polypeptide complexes and the ionization pk of the terminal amino group was demonstrated by Rabin.[8] A consideration of the effects of substituents in the peptide molecules suggested that, prior to the proton ionization, the peptide oxygen rather than nitrogen atom was coordinated to the copper ion. Kim and Martell,[4-6] on the other hand, have assigned multichelated structures to the copper complexes of di-, tri-, and tetraglycine with the peptide nitrogen atom coordinated. In order to account for the appreciably smaller stability of these complexes in solution as compared with that of the copper monoglycinate complex, it was assumed that the ligand atoms are only weakly coordinated to the metal ion.

A consideration of the enthalpy and entropy changes accompanying ion association reactions in solution can yield information concerning the nature and type of bonding between the metal ion and ligand;[11] hitherto, there have been no calorimetric investigations of the copper polyglycine systems. In the present study, potentiometric and calorimetric measurements have been made of the formation of these complexes and of the successive proton ionizations from the peptide groups.

Experimental Section

Materials. Grade A glassware and reagent grade chemicals were used, and solutions were prepared with doubly distilled water; carbon dioxide was excluded by bubbling with nitrogen. Diglycine, obtained from Mann Research Laboratories and from Nutritional Biochemical Corp., was used without further purification; it was necessary to purify the latter manufacturer's triglycine by dissolving in doubly distilled water and precipitating by the addition of absolute ethanol.[9] This procedure was twice repeated, and the product was dried to constant weight *in vacuo* over phosphorus pentoxide. Sodium and copper perchlorate solutions were prepared by neutralizing perchloric acid with sodium carbonate and copper oxide, respectively. Analysis was made by exchanging the cations for hydrogen ions on a Dowex 50 ion-exchange resin column and titrating the liberated perchloric acid; copper solutions were also analyzed by means of EDTA titrations.

Potentiometric Experiments. Emf measurements were made at 25 ± 0.02° with cells of the type

glass electrodes | solution under study | satd KCl | Hg_2Cl_2, Hg

using Beckman Type 39099-E3 and 41263 glass electrodes, a Leeds and Northrop Type K3 potentiometer, and a Victoreen picometer (Model 474) as null detector; the emf reproducibility was ±0.1 mV. Each cell incorporated a pair of glass electrodes so that any irregularity in the behavior of one of them was immediately apparent. The electrode systems were standardized before and after each experiment with NBS standard buffer solutions prepared according to Bates:[12] 0.09 M potassium chloride + 0.01 M hydrochloric acid, pH 2.07; 0.05 M potassium hydrogen phthalate, pH 4.008; 0.025 M potassium dihydrogen phosphate + 0.025 M disodium hydrogen phosphate, pH 6.865; and 0.01 M borax, pH 9.180. pH measurements were made in a nitrogen atmosphere in solutions containing the polyglycine with or without added copper perchlorate in a 1:1 metal to ligand ratio; the solutions were titrated with carbon dioxide free sodium hydroxide solution. Total concentrations of copper and polyglycine varied between 5 and 10×10^{-3} M. Sufficient sodium perchlorate was added to maintain an ionic strength to within 1% of 0.1 M over the entire titration. pH ($= -\log a_{H^+}$) values were converted to hydrogen ion concentration by using an f_{H^+} actvity coefficient value (0.7815) calculated from the Davies equation.[13]

Calorimetric Experiments. The calorimeters and general experimental techniques were developed from those described previously.[11,14] The working volume of the twin differential calorimeters was 100–150 ml, and stirring was effected with vibrating glass disks (Vibromixer Model E1, Chemapec, Inc.) Four terminal heating coils, of resistance approximately 7 ohms, were constructed of enamelled 32-gauge manganin wire wound on a glass former and held in place by means of a thin coating of silicone rubber (Dow Corning Corp.). In place of the single addition mixing device previously described,[11] the calorimeters were modified so that additions of reagent could be made from a motor-driven buret (Dosimat 10-ml automatic buret, Metrohm Herisau, Switzerland). After first passing through a thin glass coil immersed in the outer thermostat, the reagent entered the lid of the calorimeter through another glass coil encased in a mercury cup. Within the calorimeters, the tube terminated in a fine J-tip to minimize free diffusion of reagent and calorimeter contents. Other modifications included the insertion through a standard taper joint of a glass "cold" finger in the calorimeters through which thermostat water or nitrogen gas could be passed in order to bring the temperature of the calorimeter contents to that of the thermostat. Down the center and through the bottom of the cold finger was sealed another narrow glass tube terminating in a fine J-tip. This tube was filled with potassium chloride solution containing a small amount of agar and was connected to an external calomel electrode prepared by the method of Hills and Ives.[15] Through a glass tube in the calorimeter lid, a slender screened glass electrode (Beckman, Type 39167) was inserted. Potentiometric measurements of the calorimeter contents could therefore be made during the enthalpy titrations by means of a Beckman Research pH meter (Model 1019). Three calorimeters, differing in their method of temperature sensing, were used in this work: (i) a matched pair of 100,000-ohm VECO glass-encased thermistors (Type A 189, Victory Engineering Co.), incorporated in the ac Wheatstone bridge described previously;[16] (ii) a similar pair of thermistors and Wheatstone bridge circuit, energized by means of a dc source consisting of a 1.35-V mercury cell connected through a 10^5-ohm "Helipot" potential divider (the out-of-balance signal, amplified by means of a dc null detector (Leeds and Northrup Model 9834) was fed to a strip chart recorder (Sargent, Model SR)); (iii) a pair of differential quartz thermometers (Hewlett-Packard, Model 2801A) with the output fed through a digital-analog converter (Hewlett-Packard, Model 580A), to a chart recorder (Sargent, Model SR).

In a typical experiment, the polyglycine was weighed into the calorimeter dewar together with copper perchlorate solution, and sufficient sodium hydroxide was added to attain the desired pH. The ionic strength was adjusted to 0.1 M with sodium perchlorate. The temperature was brought to that of the thermostat by circulating thermostat water through the cold fingers for 1–3 hr, and the calorimeters were left to equilibrate overnight. After the attainment of a steady base line as indicated on the chart recorder, perchloric acid solution was added from the buret; the volume of each addition never exceeded 1.0 ml. Electrical calibration was made in such a way that the amount of heat used was as nearly identical as possible with that measured in an actual experiment. Successive reagent additions and electrical calibrations were continued, and the temperature of the contents of the calorimeters was periodically restored to that of the thermostat by passing thermostated water or nitrogen gas through the cold fingers. Typically, temperature

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Table I. Dissociation Constants of Di- and Triglycine at 25°

Diglycine				Triglycine			
Ref	I, M	pk_1	pk_2	Ref	I, M	pk_1	pk_2
This work	0.10	3.08 ± 0.01	8.09 ± 0.01	This work	0.10	3.18 ± 0.01	7.87 ± 0.01
17, 18	→0	3.139	8.264	6[b]	0.1	3.27	7.90
19	0.16	3.19	8.13	20	0.15	...	8.02
4	0.10	3.18	8.04[a]	21	...	3.24	8.20
				9	0.16	3.30	7.96

[a] This value was measured at 25.6°. [b] Temperature 24.9°.

Table II. Thermodynamic Functions for Proton Dissociation Reactions Involving Copper Polyglycines (25°, I = 0.1 M)

Dissociation reactions	ΔG, kcal mole^{-1}	ΔH, kcal mole^{-1}	ΔS, cal deg^{-1} mole^{-1}
	Diglycines		
(1) $H_2GG^+ \rightleftharpoons HGG + H^+$	+4.20 ± 0.01	(0.032 ± 0.016)[a]	−13.9 ± 0.6
(2) $HGG \rightleftharpoons GG^- + H^+$	+11.03 ± 0.01	+10.6 ± 0.1 (10.5 ± 0.2)[b]	−1.5 ± 0.4
(4) $CuGG^+ \rightleftharpoons CuA + H^+$	+5.54 ± 0.01	+6.9 ± 0.2	+4.5 ± 0.7
	Triglycines		
(1) $H_2GGG^+ \rightleftharpoons HGGG + H^+$	4.34 ± 0.02	+0.2 ± 0.1	−13.9 ± 0.6
(2) $HGGG \rightleftharpoons GGG^- + H^+$	10.73 ± 0.01	+10.1 ± 0.1	−2.1 ± 0.4
(8) $CuGGG^+ \rightleftharpoons CuA + H^+$	6.90 ± 0.01	+7.5 ± 0.2	2.0 ± 0.7
(9) $CuA \rightleftharpoons CuB^- + H^+$	9.25 ± 0.02	+7.4 ± 0.2	−6.2 ± 0.8

[a] Value from temperature coefficient of k_a, 5–50° (ref 18). [b] Value from temperature coefficient of k_b, 10–40°: J. Vaissermann and M. Quintin, *J. Chim. Phys.*, **63**, 731 (1965).

changes of the order of 5×10^{-3}° per addition were measured with a sensitivity of about 5×10^{-5}°. ΔH values for the dissociation of the free ligands were measured by means of similar titrations in the absence of copper perchlorate.

All calculations were done with the aid of an IBM 7044 computer.

Results and Discussion

It was first necessary to determine the thermodynamic functions for the successive proton dissociations of the polyglycine, H_2L^+, ligand molecules

$$H_2L^+ \rightleftharpoons HL^{\pm} + H^+ \qquad k_1 \qquad (1)$$

$$HL^{\pm} \rightleftharpoons L^- + H^+ \qquad k_2 \qquad (2)$$

in which L^- represents GG^- (diglycine anion) or GGG^- (triglycine anion). Potentiometric measurements of hydrogen ion activities were made on the addition of sodium hydroxide and of perchloric acid to the polyglycine at a constant ionic strength of 0.10 ± 1% M. Concentrations of ionic species were calculated in the usual manner from mass balance and electroneutrality expressions,[11] and the values of the dissociation constants at 25° are given in Table I. Each value is the mean of a minimum of 30 sets of data, and the standard deviations are also given in Table I together with the values available in the literature.[17-21] Comparison with the results of the present work is made difficult by the absence of information concerning the choice of activity coefficient, f_1, at a particular ionic strength for the conversion of pH values to concentration. The thermodynamic pk_2 = 8.264 for diglycine measured by King,[18] when converted to I = 0.1 M with f_1 = 0.7815, becomes 8.05, in good agreement with the present results.

The results of calorimetric measurements of the proton dissociation reactions of the ligand molecules are summarized in Table II. Agreement with the results of other workers for HGG is seen to be good.

In the presence of copper ions with a 1:1 copper to ligand ratio, the following equilibria may be formulated for diglycine[5]

$$Cu^{2+} + GG^- \rightleftharpoons CuGG^+ \qquad K_1 \qquad (3)$$

$$CuGG^+ \rightleftharpoons CuA + H^+ \qquad k_a' \qquad (4)$$

$$CuA \rightleftharpoons CuAOH^- + H^+ \qquad k_b' \qquad (5)$$

$$CuA + CuAOH^- \rightleftharpoons Cu_2A_2OH^- \qquad K_D \qquad (6)$$

and for triglycine

$$Cu^{2+} + GGG^- \rightleftharpoons CuGGG^+ \qquad K_1 \qquad (7)$$

$$CuGGG^+ \rightleftharpoons CuA + H^+ \qquad k_a' \qquad (8)$$

$$CuA \rightleftharpoons CuB^- + H^+ \qquad k_b' \qquad (9)$$

(17) E. J. King, "Acid-Base Equilibrium," The Macmillan Co., New York, N. Y., 1965.
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(20) N. C. Li and M. C. M. Chen, *ibid.*, **80**, 5678 (1958).
(21) H. Dobbie and W. O. Kermack, *Biochem. J.*, **59**, 257 (1955).

Table III. Copper(II)–Polypeptide Equilibrium Constants

Log K_1	pk_a'	pk_b'	Log K_D	I	Temp, °C	Ref
			Diglycine			
5.56 ± 0.01	4.06 ± 0.01	9.29 ± 0.02	2.12 ± 0.03	0.1 ($NaClO_4$)	25.0	This work
5.42 ± 0.02	4.38 ± 0.02	9.52 ± 0.02	2.07 ± 0.02	1.0 (KCl)	24.9	5
4.96	3.90	9.37	2.30	0.16 ($NaNO_3$)	25	19
5.88	4.25	9.65	...	...	20	21
5.50	4.30	...	...	0.16 (KCl)	25	*a*
			Triglycine			
5.04 ± 0.01	5.06 ± 0.01	6.78 ± 0.02		0.1 ($NaClO_4$)	25.0	This work
5.5 ± 0.1	5.4 ± 0.1	6.63 ± 0.02		0.1 (KNO_3)	24.9	6
4.80	5.10	6.89		0.16 ($NaNO_3$)	25	9
5.3	5.2	7.0		...	20	*b*

[a] G. F. Bryce, J. M. H. Pinkerton, L. K. Steinrauf, and F. R. N. Gurd, *J. Biol. Chem.*, **240**, 3829 (1965). [b] H. Dobbie and W. O. Kermack, *Biochem. J.*, **59**, 246 (1955).

in which the successively deprotonated species are derived from $GG^- = HA^-$ for diglycine and $GGG^- = HA^- = H_2B^-$ for triglycine. Although literature values are available for the equilibrium constants of the above reactions, they refer to differing conditions of ionic strength, supporting electrolyte, and temperature. It was considered desirable to determine their values under the same conditions as those used in the calorimetric experiments. To this end, potentiometric determinations of pH were made at 25° in solutions containing copper perchlorate, polyglycine, sodium hydroxide, and sodium perchlorate at a total ionic strength of 0.10 *M*. Within the pH ranges 3.9–5.1 for diglycine and 4.3–5.5 for triglycine, the relevant equilibria were limited to (3) and (4) for copper diglycine and (7) and (8) for copper triglycine. Concentrations of ionic species were calculated by the use of eq 10–15.

$$[L^-] = (B + [H^+] - [OH^-] - T_L\Omega)/(\gamma - \alpha\Omega) \quad (10)$$

$$\Omega = (1 + 2k_a'[H^+]^{-1})/(1 + k_a'[H^+]^{-1}) \quad (11)$$

$$\gamma = 1 - k_1k_2[H^+]^2 \quad (12)$$

$$\alpha = 1 + [H^+]/k_1 + [H^+]^2/k_1k_2 \quad (13)$$

$$[Cu^{2+}] = (1 - \bar{n})T_M \quad (14)$$

$$[CuL^+] = \bar{n}T_M/(1 + k_a'[H^+]^{-1}) \quad (15)$$

In these equations, T_M is the total copper ion and T_L the total ligand concentration, B represents the total concentration of base added, and $\bar{n}$ is the formation function, $(T_L - \alpha[L^-])/T_M$. K_1 values were calculated from the potentiometric results by substituting a number of selected values of k_a' in eq 10. A plot of the standard deviations in the resulting K_1 as a function of chosen k_a' resulted in parabolas with minima corresponding to the best values. Further refinement of K_1 was then made by repeating the calculations with a series of pk_a' values in increments of 0.005 around the minimum in the standard deviation curve. The data are summarized in Table III together with the standard deviations; each value is the mean of at least 40 determinations. The values of k_b' and the diglycine K_D were calculated in a similar manner from data at higher pH using the values of k_a' and K_1 obtained as described above. In agreement with Martell's findings, it was necessary to introduce the dimerization, K_D (equilibrium 6), in order to obtain a constant value of k_b' in the copper diglycine case.

The agreement between the equilibrium constants and those obtained by other workers (Table III) is satisfactory in view of the differing conditions involved in their determination and the neglect of the K_D value in one of the cases.[21] The excellent constancy of the present values serves as strong supporting evidence for the validity of the assumed ionic species in the solutions. There is little doubt that the various deprotonated species have to be taken into account in the pH ranges studied. Perrin[22] has recently proposed a method for the calculation of the concentrations of ionic species as a function of pH in mixed systems containing copper ions, triglycine, and three other ligands but failed to recognize the existence of CuA and CuB^- at the two pH values considered, 6.0 and 8.0. The assumption that only $CuGGG^+$ and $Cu(GGG)_2$ are formed under these conditions is an unjustified oversimplification.

Enthalpy titrations were made by the addition of perchloric acid to solutions containing copper perchlorate, polyglycine, and sodium hydroxide together with sufficient sodium perchlorate to maintain the ionic strength within 1% of 0.10 *M*. The pH ranges covered were 3.9–5.1 for the diglycine and 4.3–5.5 (ML^+ and MA) and 6.0–7.5 (ML^+, MA, and MB) for triglycine complexes. The concentrations of ionic species in solution at each stage of the titration were calculated from the expressions for the concentrations of free metal ion

$$[M^{2+}] = T_M/(1 + K_1[L^-] + K_1k_a'[L^-]/[H^+]) \quad (16)$$

and free ligand anion

$$[L^-] = -\tfrac{1}{2}(\alpha + \beta[T_M - T_L])(\alpha\beta)^{-1} + \frac{1}{2}\{(\alpha + \beta[T_M - T_L])^2(\alpha\beta)^{-2} + 4T_L/\alpha\beta\}^{1/2} \quad (17)$$

in which T_M = total copper ion concentration and $\beta = K_1(1 + k_a'/[H^+])$. The measured heat changes,

(22) D. D. Perrin, *Nature*, 206, 170 (1965).

Table IV. Thermodynamic Functions for the Association of Copper with Glycine and Polyglycines (25°, I = 0.10 M)

	Association reactions	ΔG, kcal mole^{-1}	ΔH, kcal mole^{-1}	ΔS, cal deg^{-1} mole^{-1}
	$Cu^{2+} + G^- \rightleftharpoons CuG^+$	-11.71 ± 0.01	-6.76 ± 0.04[a]	$+16.6 \pm 0.3$
	$CuG^+ + G^- \rightleftharpoons CuG_2$	-9.47 ± 0.02	-6.89 ± 0.1[a]	$+8.7 \pm 0.4$
(3)	$Cu^{2+} + GG^- \rightleftharpoons CuGG^+$	-7.58 ± 0.01	-6.1 ± 0.2	$+4.97 \pm 0.7$
(7)	$Cu^{2+} + GGG^- \rightleftharpoons CuGGG^+$	-6.87 ± 0.01	-6.3 ± 0.2	$+1.9 \pm 1.0$

[a] S. Boyd, J. R. Brannan, H. S. Dunsmore, and G. H. Nancollas, *J. Chem. Eng. Data*, **12**, 601 (1967).

Q_{exptl}, corrected for the measured heat of dilution of the perchloric acid into 0.1 M $NaClO_4$, may be expressed in terms of the heats of formation in solution of the species represented by the subscripts in the equation

$$Q_{exptl} = Q_{H_2O} + Q_{HL} + Q_{H_2L} + Q_{CuA} + Q_{CuL} \quad (18)$$

in which charges have been omitted for clarity. The changes in the concentrations of ionic species from point to point in the enthalpy titrations may be used to calculate the corresponding Q values from the expressions

$$Q_{H_2O} = \Delta H_{H_2O}(-\Delta[OH^-])$$

in which the heat of formation of water, ΔH_{H_2O} = -13.49 kcal mole^{-1} at an ionic strength of 0.1 M (calculated from $\Delta H°_{H_2O}$ = -13.34 kcal mole^{-1} at $I \rightarrow 0$[11]); $Q_{HL} = \Delta H_{HL}(\Delta[HL] + \Delta[H_2L])$ and $Q_{H_2L} = \Delta H_{H_2L}(\Delta[H_2L])$. Remembering that $Q_{CuL^+} = \Delta H_{CuL^+}(-\Delta[Cu^{2+}])$ and $Q_{CuA} = \Delta H_{CuA}(\Delta[CuA])$, eq 18 becomes

$$\Delta H_{CuL^+} - \frac{\Delta[CuA]}{\Delta[Cu^{2+}]}\Delta H_{CuA} = -\frac{(Q_{CuL^+} + Q_{CuA})}{\Delta[Cu^{2+}]} \quad (19)$$

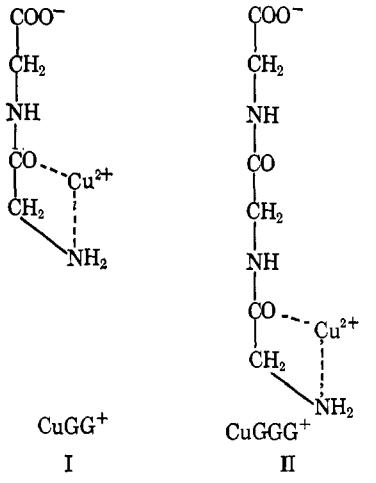
Values of ΔH_{CuL^+} and ΔH_{CuA} were obtained by least-squares procedures from the intercepts and slopes, respectively, of linear plots of $(Q_{CuL^+} + Q_{CuA})(\Delta[Cu^{2+}])^{-1}$ against $(\Delta[CuA])(\Delta[Cu^{2+}])^{-1}$. Some typical plots are shown in Figure 1, and the thermodynamic functions are given in Tables II and IV together with the mean deviations. In the case of copper triglycine, enthalpy changes accompanying reaction 9 were determined from titration data in the pH range 6.0–7.5 using the known ΔH for reactions 7 and 8; the value is also given in Table II.

There are few thermodynamic data with which to compare the present results. Murphy and Martell[10] made potentiometric titrations of copper triglycine at three temperatures between 0.35 and 48.80° using 1:1 and 2:1 molar ratios of ligand to metal. The ΔH values quoted, +4.5 for reaction 8 and +4.3 for reaction 9, are, however, in serious disagreement with the respective values +7.5 and +7.4 given in Table II for these equilibria. In view of the difficulty of obtaining enthalpy changes from temperature coefficients of the equilibrium constants,[11] we feel that the present calorimetric values are more reliable. Recently, Skinner has published a microcalorimetric study of a number of metal amino acid and peptide complexes including copper glycine and copper diglycine.[23] Heat measurements were made upon the addition of trace volumes (100–200 μl) of copper nitrate solution (0.015–0.02 M) to much larger volumes (15 ml) of the sodium salt of the ligand (0.01 M) in 0.1 M potassium nitrate. In the copper glycine case, the main component under the conditions of the microcalorimetry experiments was the bisglycinato complex CuG_2. The value obtained by Skinner for the enthalpy of formation, -13.8_2 kcal mole^{-1}, is in excellent agreement with that obtained by conventional calorimetric methods, -13.65 kcal mole^{-1} (Table IV and ref 24). For the copper diglycine complex formation, however, Skinner gives a composite value of ΔH = -16.5 kcal mole^{-1} and attributes this to the formation of CuA(OH) and CuA_2 in the ratio 7:3. The justification for this conclusion is based upon the assumption that the value for the enthalpy of formation of a "typical" copper–nitrogen bond is equal to one-quarter of the enthalpy of formation of the copper bisethylenediamine complex. In the microcalorimetric experiments, the absence of pH data made it impossible to calculate the concentrations of the complex species present. No account was taken of the fact that at the working pH of about 10, species such as CuA are produced through the initial formation and subsequent deprotonation of $CuGG^+$. Although Skinner reports the ΔH in units of kcal mole^{-1}, it is not clear to which species this value refers. In addition, at the high ligand to metal ratios involved, the species present have not been sufficiently well characterized to allow reliable analyses of such enthalpy data.

The thermodynamic data in Table IV exhibit a number of striking features. The substantial decrease in stability of the polyglycine complexes, $CuGG^+$ and $CuGGG^+$, as compared with the monoglycinate, CuG^+, is seen to be essentially an entropy effect. The exothermic enthalpies of formation are remarkably constant for all three complexes, indicating that di- and triglycine are bound in bidentate structures similar to that for glycine itself and not in the multichelated structures proposed by Kim and Martell.[5,6] It is likely that the structures in solution resemble those in the solid state[2,3] with the copper bonded to the terminal NH_2 and the oxygen in the first peptide group in the five-membered chelate ring structures I and II. The bond between the metal ion and the peptide oxygen atom will be essentially electrostatic in character, leading to ΔH values similar to that for CuG^+ formation. Structures, based upon the results of infrared measurements in which the peptide nitrogen atom is bound to the copper ion in CuL^+, have been proposed by Kim and

(23) H. A. Skinner, *Trans. Faraday Soc.*, **63**, 1136 (1967).

(24) S. Boyd, J. R. Brannan, H. S. Dunsmore, and G. H. Nancollas, *J. Chem. Eng. Data*, **12**, 601 (1967).



Martell.[5,6] Although such spectral measurements add strong supporting evidence for peptide hydrogen ionization and metal–nitrogen bond formation at higher pH, the conclusions concerning the nature of the bonding in CuL^+ at lower pH are open to question since the solutions contained appreciable quantities of CuA as well as CuL^+. The infrared data can be interpreted equally well by assuming that the interaction between the copper ion and the peptide group occurs through the peptide carbonyl oxygen rather than with the peptide nitrogen as suggested by Kim and Martell.[5,6] The observed spectral shifts of the peptide carbonyl bands $\sim$1675 to $\sim$1630 cm^{-1} at pH $\sim$4 are not inconsistent with the formation of $-NHC{=}O\cdots Cu$. Further evidence for copper–peptide oxygen bonding in aqueous CuL^+ is based upon structural considerations. Thus binding of the nitrogen atom of the peptide group would require not only a considerable distortion of the polyglycine ligand molecule but would also be expected to result in more exothermic enthalpies of formation than those given in Table IV. The planarity and resonance energy of the peptide group are not sacrificed if the copper atom is bound at the peptide oxygen. Rabin[8] has presented convincing arguments for the nonparticipation of the peptide nitrogen atom based on the effects of substituents upon the stabilities of a number of copper peptide complexes; the data in Table IV add supporting evidence for this conclusion.

In the formation of a complex by the association of two oppositely charged ions, a moderately large and unfavorable negative entropy change would be expected, reflecting the disappearance of a particle in the system. In most cases of metal complex formation, however, there is a favorable positive entropy change resulting from the breakdown of the "iceberg" structure of coordinated water molecules around the ions and decreased orientation of solvent molecules. This release of coordinated water molecules is the most important factor determining the entropy of association. In the structures I and II, the carboxyl groups are not bound to the copper ion and the resulting charge separation and retention of some solvent ordering will account for the small observed ΔS values for the formation of CuL^+ decreasing in the order $\Delta S(CuGG^+) > \Delta S(CuGGG^+)$. In marked contrast, the larger positive ΔS value for the formation of the monoglycinate complex reflects not only a more effective neutralization of charge through short-bond formation in the xy plane of coordination

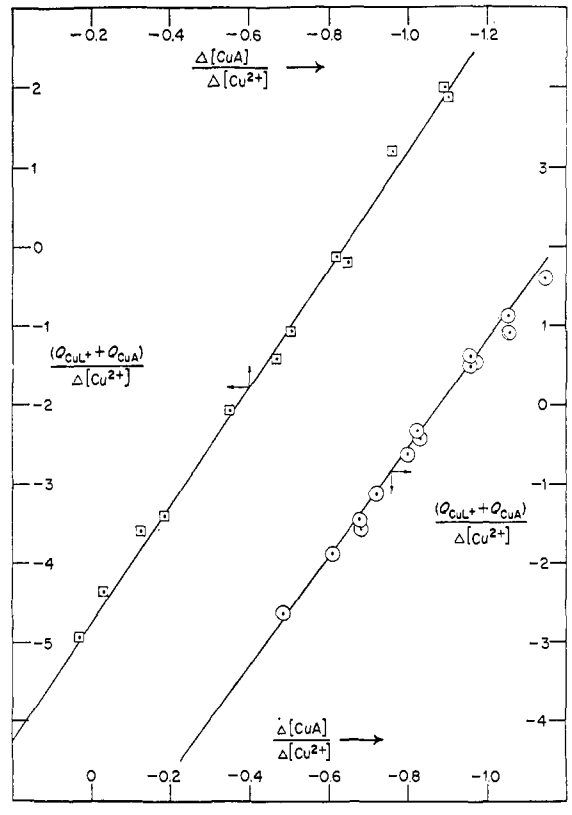


Figure 1. Plots of $(Q_{CuL^+} + Q_{CuA})(\Delta[Cu^{2+}])^{-1}$ against $(\Delta[CuA])\cdot(\Delta[Cu^{2+}])^{-1}$: ⊙ refers to copper diglycine, ⊡ to copper triglycine.

but also a greater degree of tetragonal distortion of the octahedral symmetry and freeing of the axial water molecules.[25]

Thermodynamic functions (Table II) for the proton dissociation reactions 4 and 8 indicate that the labilization of the peptide hydrogen in the complexes as compared with the ligand (reaction 2) is largely an enthalpy effect. Although there are no enthalpy data available for the dissociation of a hydrogen from an unbound peptide group, the values would be expected to be even more endothermic than the enthalpy changes accompanying the proton dissociation from the zwitterions $HL^{\pm}$. It is seen that the ΔH values for reactions 4 and 8 are considerably less endothermic than those for reaction 2. Assuming multichelated structures for the CuA species, their enthalpies of formation reflect not only the proton dissociation but also the formation of bonds between the metal, the nitrogen atom of the peptide group, and the carboxyl oxygen atoms.

Although a detailed discussion of small changes in thermodynamic properties for series of proton dissociation reactions is not possible at the present time,[17] it is interesting to examine the gross features of the data in Table II. Three important factors must be considered in discussing the ΔS values for dissociation reactions: (i) the production of an ionic species, (ii) changes in solvent structure associated with the cospheres of the ions,[26] and (iii) changes in the internal freedom of the molecules. If the first factor was the only important consideration, we would expect a mod-

(25) A. McAuley, G. H. Nancollas, and K. Torrance, *Inorg. Chem.*, 6, 136 (1967).
(26) R. W. Gurney, "Ionic Processes in Solution," McGraw-Hill Book Co., Inc., New York, N. Y., 1953.

erately large and positive ΔS. However factor ii, describing the environmental changes, is usually of overriding importance. For isoelectric reactions, ΔS for changes in solvent ordering will be small, but, where an anionic and cationic species are produced, moderately large negative entropy changes will result. The magnitude will depend upon the relative hydration of the ionic species. Normally factor iii will make a rather small contribution to ΔS except in cases where large ligand molecules are involved. In Table II it is seen that the ΔS values for reaction 1 are considerably more negative than those for reaction 2. HGG and HGGG are zwitterions with appreciable solvent ordering properties, especially at the $-NH_3^+$, and also a degree of internal structure. The differences in ΔS can thus be explained on the basis of factors ii and iii above. The change in solvent ordering accompanying reaction 2 will be expected to be smaller than for reaction 1 and, also in the former case, there will be an increase in the internal freedom of the molecules in going from $HL^{\pm}$ to L^-. The difficulty of removing a proton from a zwitterion as compared with a positively charged ion is reflected by the more endothermic enthalpy changes for reaction 2. In these reactions, the fields associated with an anion and a cation have to be created.

In reactions 4 and 8, the proton dissociates from the copper complexes CuL^+ which with its charge separation (structure I) will retain considerable solvent ordering properties. The entropy decrease predicted by factor ii above will therefore be moderately small. The positive ΔS accompanying an increase in the number of particles will be offset to some extent by the loss of freedom of the ligands as they form the multichelated structures CuA from CuL^+. The last effect is larger for triglycine than for diglycine and it is seen in Table II that ΔS for reaction 8 is less positive than that for reaction 4. For reaction 9, the fields associated with the oppositely charged ions CuB^- and H^+ have to be created, and the more negative ΔS in Table II results. It is interesting to note that this entropy difference between reactions 9 and 8 accounts entirely for the more difficult proton dissociation from CuA as compared with that from $CuGGG^+$.

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