METAL CHELATES OF GLYCINE AND GLYCINE PEPTIDES*

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Since the advancement by Smith et al. (1-4) of the theory that metal ions are chelated simultaneously to enzyme protein and substrate in various polypeptidases, the nature of metal binding to peptides and proteins has become an important as well as an interesting problem. The chelate structures proposed by Smith (3), and later somewhat revised (5, 6), indicate that the metal is bound, at least to the substrate and possibly to the enzyme protein, through the polar groups in two or more adjacent peptide linkages. The affinity of binding, but not the specific groups involved, can be determined for the interaction of metal ions with proteins. It would, therefore, be of interest to study the affinity of the binding to simple peptides for which the nature of the groups bound to the metal ion can be specified with considerable certainty. The accumulation of sufficient data of this type may make it possible to deduce the types of linkages involved in metal-protein combinations. As an approach to this general problem, the present paper describes an investigation of the interactions of glycine peptides and glycine with Mg(II), Mn(II), and Cu(II) ions.

EXPERIMENTAL

Method—The experimental method consisted of potentiometric measurements of pH by a technique similar to that described by Chaberek and Martell (7). The experimental consisted of titration by standard potassium hydroxide of a solution containing the ligand with or without the metal ion. As supporting electrolyte, potassium chloride was added so as to produce an ionic strength of 0.09. All titrations were carried out at three temperatures, 0.35° ± 0.05°, 30.00° ± 0.01°, and 48.80° ± 0.10°, and at both 1:1 and 2:1 molar ratios of ligand to metal ion.

Materials—Carbonate-free potassium hydroxide was prepared by the method of Schwarzenbach and Biedermann (8). The solutions of potassium chloride and the chlorides of magnesium, manganese, and copper were prepared from J. T. Baker Chemical Company analyzed reagents. The divalent metal solutions were standardized by alkalimetric titration in the presence of acid salts of ethylenediaminetetraacetic acid. The

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glycine, obtained as c.p. material from the Fisher Scientific Company, Eimer and Amend Division, was recrystallized two times from distilled water. Pure glycylglycine and glycylglycylglycine were prepared by A. R. Manyak (9).

Procedure—The following procedure was employed in all experimentals, except that the metal ion was omitted for those designed to determine the second dissociation constant of the ligand. About $1.5 \times 10^{-3}$ mole of ligand was introduced into the titration cell, and sufficient potassium chloride solution and water were added to bring the ionic strength to the desired value. An inert atmosphere was maintained by passing nitrogen first through a potassium chloride solution at the same ionic strength and then through the titration cell.

The pH meter was calibrated with a cell consisting of hydrogen and silver-silver chloride electrodes under the conditions used in a typical titration. A calibration curve covering the range of the pH meter was thus obtained. In the experimentals, pH readings were taken after the addition of small quantities of standard base until a high pH value was obtained or precipitation of the metal hydroxide occurred.

Results

Potentiometric Titrations—Sample potentiometric measurements of corrected pH values versus moles of base added per mole of ligand present are given in Figs. 1, 2, and 3. Only data taken at 0.35$^\circ$ are shown, since the results obtained at the other temperatures are similar, with corresponding points differing only by a fraction of a pH unit. The data thus obtained were used in the calculations of equilibrium constants and thermodynamic quantities associated with the chemical reactions which occur.

Stoichiometric Relationships from Titration Curves—Before it is possible to set up relationships for equilibrium constants, it is first necessary to decide what reactions probably occur under the experimental conditions. This was done by correlating the end points of reaction and the positions of the buffer regions in the titration curves with the stoichiometry of the solution constituents and the known structures of the ligands.

The titration curves in Fig. 1 have inflection regions (regions of increased slope) at $a = 1$ for all metals concerned and for both 1:1 and 2:1 ratios of ligand to metal ion. Thus, regardless of the metal ion and the formula of the chelate, 1 mole of hydrogen ion is displaced from each mole of ligand as the metal chelate is formed. Since the inflections at $a = 1$ can be taken as the completion of a chemical reaction, chelate formation with glycine may be summarized by the following equations:

\[
\begin{align*}
M^{2+} + HG^+ + OH^- & \rightarrow MG^+ + H_2O \\
MG^+ + HG^+ + OH^- & \rightarrow Mg_2 + H_2O
\end{align*}
\]
where $M^{+2}$ and $HG^+$ represent a metal ion and glycine, respectively. When the ratio of ligand to metal is 2:1, both reactions may be assumed to occur in the buffer region between $a = 0$ and $a = 1$. The overlapping of the two reactions prevents the occurrence of an inflection region at $a = 0.5$, which would be characteristic of the reactions taking place in two separate steps.

The glycylglycine curves of Fig. 2 with Mg(II) and Mn(II) ions are similar to the corresponding glycine curves, and the reactions may be summarized in the same way. The titration curve obtained for a 1:1 molar ratio of ligand to copper(II) ion has a long flat buffer region in which 2 moles of base per mole of ligand are required. Thus 2 hydrogen ions are neutralized for every molecule of metal chelate formed. This reaction may be summarized by the chemical equation:

$$Cu^{+2} + H_2GG + 2OH^- \rightarrow CuGG + 2H_2O$$

where $H_2GG$ represents neutral glycylglycine with 2 protons potentially displaceable by metal ions. For the 2:1 titration curve, with half the
amount of copper(II) ion present, the low buffer region requires half as much base. Thus the same reaction may be assumed to occur with half of the ligand present in the solution. The further interactions which occur at higher pH are rather more complex in nature and can be interpreted in a number of ways to be discussed below.

The glycylglycylglycine curves of Fig. 3 also show a single proton displacement with Mg(II) and Mn(II) ions, so that the compounds formed again resemble the glycinate derivatives. On the other hand, the 1:1 ligand to Cu(II) ion curve has a single buffer region in which 3 moles of base are neutralized. Hence, the over-all reaction may be summarized as a 3 proton reaction, according to the following chemical equation:

$$\text{Cu}^{+2} + H_3\text{GGG} + 3\text{OH}^- \rightarrow \text{CuGGG}^- + 3\text{H}_2\text{O}$$

When the ratio of ligand to metal ion is 2:1, the titration curve rises steadily and with only slight variations of slope, until 2 moles of base per mole of metal ion have been added, whereupon a final inflection rise occurs at high pH (between 10 and 11). This may be interpreted in terms of a series of four overlapping neutralization steps during which a 2:1 chelate is formed and 4 hydrogen ions are eliminated. The over-all reaction may be expressed as

$$\text{Cu}^{+2} + 2H_2\text{GGG} + 4\text{OH}^- \rightarrow \text{Cu(HGGG)}_2 + 4\text{H}_2\text{O}$$

Alternatively, the 2:1 titration may be interpreted in terms of the 1:1 reaction which results in the formation of CuGGG, plus subsequent neutralization of excess ligand at a high pH.
Calculation of Acid Dissociation Constants—The acid dissociation constants of the ligands were calculated from the titration data by a direct algebraic method. The equation used for all nine sets of data involved (three temperatures for each of three ligands) is

\[ k_2 = \frac{[\text{H}^+][\alpha \text{CA} + [\text{H}^+] - [\text{OH}^-] - [\text{OH}^-] + [\text{OH}^-]}}{(1 - a) \text{CA} - [\text{H}^+] + [\text{OH}^-]} \]

where [ ] represents the concentration of ions, \( a \) = the moles of base added per mole of ligand, \( \text{CA} \) = the total initial concentration of ligand species, and \( k_2 \) = the "concentration" constant, defined by

\[ k_a = \frac{[\text{H}^+][\text{Z}^-]}{[\text{HZ}^\pm]} \]

where \( \text{HZ}^\pm \) represents the neutral (dipolar) form of the ligand.

Calculation of Chelate Stability Constants—The first and second successive chelate formation constants of glycine were calculated by a direct algebraic method. These two equilibrium constants are defined by the relationships

\[ K_1 = \frac{[\text{MZ}^+]}{[\text{M}^\text{Z}^2][\text{Z}^-]} \]

\[ K_2 = \frac{[\text{MZ}^2]}{[\text{MZ}^3][\text{Z}^-]} \]

The chelate formation constants can be expressed in terms of directly measurable quantities according to the following equations:

\[ K_1 = \frac{C_A - X[Z^-]}{[Z^-][(C_M - C_A) + X[Z^-]^2]} \]

\[ K_2 = \frac{C_A - X[Z^-] + k_1[Z^-]C_A - X[Z^-] - C_M)}{k_1[Z^-]^2X[Z^-] + 2C_M - C_A) \]

where \( X = \frac{[\text{H}^+]}{k_2} + 1 \), \( C_M \) = the total concentration of metal species, and the other terms are as previously defined.

It is evident that the sum of the terms \([\text{MZ}^+]\) and \(2[\text{MZ}^2]\) represents the total moles of the ligand bound. This quantity, divided by \( C_M \), is therefore the average number of moles of ligand bound per mole of metal ion, a quantity known as the formation function, and is designated by \( \bar{n} \) in the Bjerrum (10) method of calculation. According to this method, the ligand anion concentration is equivalent to the reciprocal of the formation constant at \( \bar{n} = 0.5 \). In the region of the titration curve where \( \bar{n} \) is less than 0.5, the concentration of \( \text{MZ}^2 \) may be neglected. Under these conditions,
$K_1$ may be expressed by the relationship

$$K_1 = \frac{n}{(1 - n)[Z^-]}$$

Thus, the condition $n = 0.5$ and a graphic plot required under the simpler Bjerrum method are not needed for evaluation of the formation constant.

In the titrations of peptides with cupric ions, the number of hydrogen ions neutralized is greater than the 1 hydrogen ion which can be neutralized in the absence of polyvalent metal ions. Thus, the peptides may be considered as polyprotic acids which dissociate in the presence of cupric ion in the following manner:

$$\text{Cu}^{++} + \text{H}_2\text{GGG}^+ \rightleftharpoons \text{CuH}_2\text{GGG}^+ + \text{H}^+ \quad K' = K_1k_2$$

$$\text{CuH}_2\text{GGG}^+ \rightleftharpoons \text{CuHGGG} + \text{H}^+ \quad K_{2a} = \frac{[\text{CuHGGG}][\text{H}^+]}{[\text{CuH}_2\text{GGG}]}$$

$$\text{CuHGGG} \rightleftharpoons \text{CuGGG}^- + \text{H}^+ \quad K_{1a} = \frac{[\text{CuGGG}][\text{H}^+]}{[\text{CuHGGG}]}$$

where $K'$ is equivalent to the product of the first chelate formation constant, $K_1$, and the acid dissociation constant of the ligand, $k_2$. The same equations apply to the determination of $K'$ and $K_{1a}$ for glycylglycine and copper.

The calculation of these equilibrium constants is conveniently carried out by means of a modified Bjerrum method applied in such a way that the hydrogen ion is considered the ligand, and its stepwise association with the most basic form of the chelates (CuGGG$^-$ and CuGG) is determined. Thus, $n$ is defined as the average number of moles of hydrogen ions bound per mole of metal chelate. When $n$ is plotted versus pH, it is assumed that, as $n = 0.5$, half of the metal chelate is in the form, CuGGG$^-$ or CuGG, and that the remainder is in the monoacid form, CuHGGG and CuHGG$^+$. Under these conditions the equilibrium constant, $K_{1a}$, is equal to the hydrogen ion concentration. Similarly, for glycylglycylglycine-Cu (II), the value of $K_{2a}$ is equivalent to the hydrogen ion concentration at $n = 1.5$.

When the ratio of peptide to metal ion is 2:1, there are several possible interpretations of the titration curves, as indicated above. Under these conditions the data available scarcely justify one to assume more than the formation of the 1:1 chelate demonstrated as present for solutions in which the ratio of reactants is 1:1. Therefore, the 2:1 titrations were not used for the calculation of the association constants involving more than 1 mole of ligand per mole of metal ion.

Equilibrium Constants—The equilibrium constants calculated by the
methods described above are given in Table I for each of the three temperatures employed. In the case of glycine, the first formation constants $K_1$ calculated from two sets of conditions, i.e. in which the ratios of ligand to metal ion were 1:1 and 2:1, are given for comparison.

The acid dissociation constants and formation constants listed in Table I cannot be compared directly with values found in the literature because of differences in formulation, temperature, and ionic strength. When the values in the literature are properly interpolated to our conditions, there is general agreement. One outstanding exception is the much lower stability of monoglycino-Mg(II) reported in this work than that reported by Monk (11). Monk’s value is subject to some doubt, however, since it is the same as the constant which he reported for the Mn(II) chelate of glycine. The affinities of α-amino acids for Mg(II) ion are generally much lower than the corresponding values for Mn(II).

<table>
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<tr>
<th>Ligand</th>
<th>Temperature °C</th>
<th>$pK_a$</th>
<th>Ratio</th>
<th>$K_1^{Mg}$</th>
<th>$K_1^{Mn}$</th>
<th>$K_1^{Cu}$</th>
<th>$K_2^{Cu}$</th>
<th>$K_1^{La}$</th>
<th>$K_2^{La}$</th>
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<td>5.38</td>
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</table>

* $z$ indicates that the value obtained was not constant.

† $K = \frac{n}{(1 - \bar{n})[A]}$ at the maximal $\bar{n}$ value obtained.
The only previously published formation constants of glycylglycine and glycylglycylglycine chelates, in which the displacement of hydrogen ions was taken into account, were reported for Cu(II) by Dobbie et al. (12). In so far as comparisons are possible, the interpolated results of the present paper are for the most part in approximate agreement with the values reported by Dobbie et al., where there is agreement in the nature of the compounds assumed to be present. Some rather fundamental differences in the composition and structures of the metal chelates formed are given under "Discussion."

**Table II**

**Thermodynamic Changes**

<table>
<thead>
<tr>
<th>Chemical conversion</th>
<th>T, °K</th>
<th>$-\Delta F^o$ (kilocalories)</th>
<th>$-\Delta H^o$ (kilocalories)</th>
<th>$\Delta S^o$ (e.u.)</th>
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</thead>
<tbody>
<tr>
<td>HG $\rightarrow$ G$^-$ + H$^+$</td>
<td>273.4</td>
<td>$-12.8 \pm 0.1$</td>
<td>$-10.3 \pm 0.2$</td>
<td>$-9.2 \pm 0.5$</td>
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<td></td>
<td>303.2</td>
<td>$-13.1 \pm 0.1$</td>
<td>$-10.2 \pm 0.4$</td>
<td>$-9.5 \pm 1$</td>
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<td></td>
<td>322.0</td>
<td>$-13.3 \pm 0.1$</td>
<td>$-10.2 \pm 0.4$</td>
<td>$-9.5 \pm 1$</td>
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<tr>
<td>H$_2$GG $\rightarrow$ HGG$^-$ + H$^+$</td>
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<td>$-10.9 \pm 0.1$</td>
<td>$-9.0 \pm 0.1$</td>
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<td>$-11.1 \pm 0.1$</td>
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<td>$3.0 \pm 0.7$</td>
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<td>H$_3$GGG $\rightarrow$ H$_2$GGG$^-$ + H$^+$</td>
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<td>$-10.7 \pm 0.1$</td>
<td>$-10.6 \pm 0.1$</td>
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<td>CuHGGGG $\rightarrow$ CuGGG + H$^+$</td>
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<td>$-9.2 \pm 0.1$</td>
<td>$-4.3 \pm 0.2$</td>
<td>$-18.0 \pm 1$</td>
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<tr>
<td></td>
<td>303.2</td>
<td>$-9.8 \pm 0.1$</td>
<td>$-4.3 \pm 0.2$</td>
<td>$-18.0 \pm 1$</td>
</tr>
<tr>
<td>CuH$_2$GGG $\rightarrow$ CuHGG + 2H$^+$</td>
<td>273.4</td>
<td>$-7.6 \pm 0.1$</td>
<td>$-4.5 \pm 0.2$</td>
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<tr>
<td>Cu + H$_2$GG $\rightarrow$ CuGG + 2H$^+$</td>
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<td>$-9.5 \pm 0.1$</td>
<td>$-7.9 \pm 0.2$</td>
<td>$-6 \pm 1$</td>
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<td>Mn$^{++}$ + HGG$^-$ $\rightarrow$ MnHGG$^+$</td>
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**Thermodynamic Relationships**—With the aid of standard thermodynamic relationships, the temperature coefficients of the equilibrium constants given above were used to calculate the changes in thermodynamic quanti-
ties associated with the conversion of reactants to products in each reaction considered. In many cases the values of $\Delta H$ and $\Delta S$ agreed rather well for the two temperature ranges. These quantities are listed in Table II, along with the chemical conversions to which they correspond. In many other cases, the value of $\Delta H$ was seen to vary considerably with temperature. In order to be certain of obtaining valid values of $\Delta H$ and $\Delta S$ in such cases, it is necessary to have $\Delta F$ values over small ranges of temperature. The thermodynamic quantities which showed such variation are omitted from Table II. The signs of $\Delta F$, $\Delta H$, and $\Delta S$ listed are such that positive values in Table II favor the conversion from left to right.

DISCUSSION

Relative Effects of Metal Ions—The relative stabilities reported in Table I for the Mg(II), Mn(II), and Cu(II) chelates are the same for all the ligands (Cu $\gg$ Mn $>$ Mg). This is in conformity to the general order of stability of chelates of these metals with other ligands. It is interesting to note, however, that, while the orders of stability are qualitatively in agreement, such a comparison can be applied only to the first association reaction. In the formation of the peptide chelates involving the displacement of more than 1 proton per ligand, the difference between copper and the other metals is absolute, since there is no counterpart of this reaction involving the other metal ions measured.

Chelate Structures—The structures of the glycine chelates formed for 1:1 and 2:1 ratios of ligand to metal ion are represented by formulas I and II, respectively. These formulations are in conformity with the bidentate nature of the ligand and the titration data, which indicate that 1 hydrogen ion is displaced per molecule of ligand. The coordination numbers of the metal ions, based on the structures of other chelate compounds in aqueous solution, are 4 for Mg(II) and Cu(II) ions and 6 for the Mn(II) ion. Therefore, the values of $n$ and $m$ may be assumed to be 2 and 0 for the copper and magnesium chelates and to have values of 4 and 2, respectively, for the manganous compounds. Although the reaction between the second ligand and the 1:1 chelate was too weak to allow the calculation of the second formation constant, there can be little doubt that it is formed
in solution. The second chelate constant can probably be measured potentiometrically for these 2 ions in solutions of much higher concentrations than those used in the present investigation.

The binding of glycylglycine and of glycylglycylglycine to Mg(II) and Mn(II) ions, while very weak, is considerably stronger than that of ammonia or a simple alkyl amine. Hence, the existence of chelate structures is again indicated. This can mean only that the peptide linkage must be involved in coordination with the metal. The additional stability resulting from simple coordination of the end amino and carboxylate groups, with the formation of 8- and 11-membered chelate rings, would be very small and would not account for the observed stability. Therefore, structures III and IV are tentatively suggested as possible arrangements of glycylglycine and glycylglycylglycine in these chelate compounds. It is possible that the latter compounds IV are tridentate rather than tetradentate. It should be emphasized that the stabilities of these compounds are much lower than would normally be expected for tridentate chelates. This can only be attributed to the weakness of the peptide linkage as a metal coordinating group. This is probably due in turn to strong hydrogen bonding of the peptide with the solvent. The reduction of hydrogen bonding which would result from formation of coordination compounds III and IV would involve a free energy increase which would result in decreased aqueous stability of the chelate relative to the free metal ion and peptide.

The one-step reactions between the peptides and copper(II) ion involving the dissociation of 2 and 3 moles of hydrogen ion are strong evidence for chelate structures V and VI, in which a hydrogen ion is displaced from each peptide linkage. An associated water molecule is shown in structure V to account for the coordination requirement of four donor groups per copper(II) ion. A number of intermediate chelates which have been considered in the stepwise formation of (V) and (VI) probably have similar structures with additional hydrogen ions attached to the peptide linkages.
The displacement of a hydrogen ion from an amide group on chelation with a metal is a unique reaction in coordination chemistry. To our knowledge, no structures of this sort have been reported previously. Dobbie et al. (12) also found that a total of 2 and 3 protons was liberated, but did not publish the structure of the chelate compound formed. However, Dobbie and Kermack (13) later suggested that the protons are probably eliminated from the peptide linkages. The only alternatives to structures V and VI are those in which coordinated water molecules are converted to hydroxyl groups. Such a possibility can be eliminated, however, since the removal of a hydrogen ion from a water molecule coordinated to copper(II) takes place at a much higher pH (neutral or above) than the values which precede the inflection regions of the 1:1 copper curves of Figs. 2 and 3.

The titration curves in which the molar ratio of peptide to copper(II) is 2:1 are remarkably different in appearance. However, it is noted that the glycylglycine curve shows an inflection at 1 mole of base per mole of ligand, followed by further neutralization reactions at a higher pH, resulting finally in the utilization of 2 moles of base. The titration curves for the tripeptide are similar, without a definite inflection region. Dobbie et al. (12) have interpreted these curves to indicate stepwise dissociation reactions involving 2:1 chelates, and Dobbie and Kermack (13) have suggested structures corresponding to (VII) and (VIII) for these chelate compounds.

It is apparent that the 2:1 titration curves can be interpreted in a number of alternative ways. Therefore, in the absence of additional evidence, it is not justifiable to assume more than the formation of structures V and VI. More experimental work is apparently needed to establish the probability of additional interactions to give compounds of the type indicated by (VII) and (VIII).

**Thermodynamic Constants**—In general, the thermodynamic constants given in Table II for association of ligand anions with metal ions are in agreement with the qualitative predictions of Martell and Calvin (14) for
this type of reaction, in that an increase of entropy is associated with the formation of the metal chelate compounds. This increase of entropy is generally characteristic of the formation of stable metal chelate rings and generally involves a large proportion of the contribution to $\Delta F$ when a relatively large number of metal chelate rings is formed (14). On the other hand, the effect is less likely to be observed in the formation of bidentate chelates. Accordingly, the entropy effect reported for the relatively weak chelate of Mn(II) with glycylglycine and glycine is approximately zero. For the relatively stable copper(II) glycinate, however, approximately one-third of the driving force of the reaction is attributable to the entropy, while in the case of the relatively weak magnesium glycylglycylglycinate, and manganese(II) glycylglycinate, entropy changes drive the reactions against unfavorable enthalpy changes. Reactions which involve dissociation of hydrogen ions from basic substances are generally endothermic. It is interesting that formation of the copper glycylglycinate and glycylglyclyglycinate chelates are less endothermic than would be expected in view of the dissociation of 2 and 3 hydrogen ions, respectively. This is an indication of the strong energy of binding between the cupric ion and the binegative glycylglycinate (GG$^{-}$) residue (formula V) and the trinegative glycylglycylglycine (GGG$^{-3}$) residue (formula VI).
Another interesting result of the thermodynamic measurements is that metal ions which are normally weakly bound to α-amino acids, such as Mg(II) and Mn(II) ions, combine with the peptides only with a considerable expenditure of energy. This results in the unusual situation of a metal chelate, which greatly increases in stability as the temperature is increased and which would not be formed at all except for a favorable entropy change. The unfavorable enthalpy changes observed for these chelates are probably a consequence of the low enthalpy of the free ligand resulting from a high degree of hydrogen bonding with the solvent. As the temperature is increased and the hydrogen bonds are partially broken, the ligand is better able to combine with the metal ion to form a chelate compound.

Applications to Binding of Metals by Proteins—The magnitudes of the stability constants observed for the interactions of peptides with Mg(II) and Mn(II) ions do not account for the higher affinities observed between these metal ions and proteins. Hence, structures similar to formula IX proposed by Smith (3) for a metal-enzyme combination cannot account for the experimental facts. There is little doubt that metals are bound to the peptide chain through coordinating groups; however, the coordinating

\[
\begin{align*}
\text{Peptide chain} & \quad \text{Cu} \quad \text{O}^- \\
\text{NH} & \quad \text{N} \quad \text{C} \\
\text{CH} & \quad \text{O} \\
\text{CO} & \quad \text{NH} \\
\text{NH} & \quad \text{NH} \\
\text{CH} & \quad \text{CO} \\
\text{CO} & \quad \text{CHR} \\
\text{H}_2\text{O} & \quad \text{OH}_2 \\
\end{align*}
\]
groups must be those available on the side chains of the constituent amino acids, such as carboxyl and imidazole groups. Peptide linkages alone are too weak to account for the coordination of metal ions as basic as Mg(II) and Mn(II) ions, to the extent which has been observed in proteins. On the other hand Cu(II) is strongly bound to simple peptide linkages, as indicated in (X), as the result of displacement of hydrogen ions from the amide nitrogen atoms.

SUMMARY

The chelate formation constants of glycine, glycylglycine, and glycylglycylglycine with Mg(II), Mn(II), and Cu(II) ions have been determined. Normal metal chelates, in which a single hydrogen ion is displaced from the free ammonium group, are formed by glycine with all three metals and by the peptides with Mg(II) and Mn(II). The Cu(II) peptide chelates are unique in that stable chelate compounds are formed with the simultaneous displacement of a hydrogen ion from each peptide linkage. The approximate enthalpy and entropy changes for acid dissociation and chelate formation reactions are given, and evidence is presented in support of the structures assigned to the various types of metal chelates formed.

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