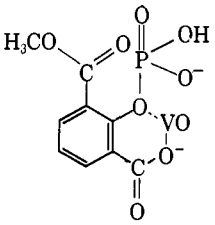
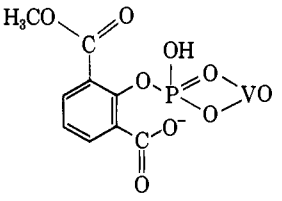

XVI


XVII

Through the formation of structures of the type indicated by XVI and XVII, the vanadyl ion would tend to withdraw electrons from the O–P bond which undergoes cleavage, thereby accelerating the reaction. However, the formation of the activated complex between vanadyl ion and the substrate would result in less interaction if the mechanism involves XVI. If the transition state is represented by XVII, the decreased rate of reaction of the monoionic form k_I, relative to that of the diionic form k_{II}, would be expected on the basis of the weaker donor effect of the adjacent methylcarboxyl group, as compared to a carboxylate ion.

Further study of the effects of pH and concentration on this reaction would be of interest. Variation of pH, for example, could indicate whether displacement of the proton of the phosphate group would give a reaction intermediate. Positive evidence on this point would favor XVII as the intermediate.

(b) **Other Catalysts.**—Free cupric ion was inert even at $-\log [H^+] = 4.00$. This is presumably due to the fact that the electronegativity of the Cu(II) ion is less than that of the vanadyl ion and would, therefore, have less affinity for the substrate.

The Cu(II)–Tiron chelate was found to be very slightly active as a catalyst even at $-\log [H^+] = 8.00$, where 70 mole % of the triionic and 30% of the diionic species are present. Since the Cu(II)–Tiron chelate has a negative charge of two, its tendency to interact with the substrate species which have charges of -3 and -4 is probably very weak as the result of electrostatic repulsion between the ligands and the substrate. Moreover, the high formation constant of this chelate ($\log K_1 = 14.53$ at 20°)[27] indicates that the metal would have only very weak affinity for an additional ligand such as the substrate.

Acknowledgment.—The authors are indebted to Dr. David Todd of Worcester Polytechnic Institute for supplying the organic phosphates and the isophthalic acid derivatives which have been used in this work.

(27) G. Schwarzenbach and A. Willi, unpublished results; J. Bjerrum, G. Schwarzenbach, and L. G. Sillén, "Stability Constants," The Chemical Society, London, 1957, part I, p. 45.

[CONTRIBUTION OF THE EASTERN RESEARCH LABORATORY OF THE DOW CHEMICAL COMPANY, FRAMINGHAM, MASSACHUSETTS, THE DEPARTMENT OF CHEMISTRY OF CLARK UNIVERSITY, WORCESTER, MASSACHUSETTS, AND THE DEPARTMENT OF CHEMISTRY, ILLINOIS INSTITUTE OF TECHNOLOGY, CHICAGO 16, ILLINOIS]

Copper(II) Chelates of Histidylhistidine and Related Compounds[1]

BY M. A. DORAN, S. CHABEREK, AND A. E. MARTELL[2]

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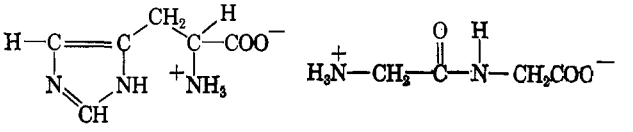
The interactions of Cu(II) ions with histidylhistidine, histidine, histamine, and glycylglycine were investigated by potentiometric and spectrophotometric techniques. Acid dissociation constants of the ligands and the stability constants of the Cu(II) chelate compounds are reported. Evidence is given for a polynuclear Cu(II)–histamine complex. Probable structures of the metal chelate compounds are suggested, and their relationship to analogous metal chelate compounds occurring in biological systems is discussed.

In recent years considerable experimental evidence has accumulated which indicates that the imidazole group of histidine may serve a number of important biological functions, such as the catalytic site for hydrolytic enzymes and as an important part of the buffering action of proteins in the physiological pH range. In view of the ease with which histidine, and in particular its imidazole group, interacts with both hydrogen ions and metal ions, it seemed worthwhile to investigate the specific acid–base and metal ion interactions of histidine (I) itself, and of its dipeptide, histidylhistidine (II). For purposes of comparison, it was decided to study analogous equilibria of related compounds of glycylglycine (III), and of histamine (IV). The Cu(II) ion was chosen because of importance in biological systems, as well as its strong coordination tendencies.

A search of the literature revealed that a limited amount of work has been reported on the metal complexes of the dipeptide, histidylhistidine. Photaki, *et al.*,[3] have observed that in the titration of an equimolar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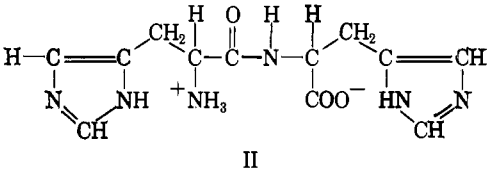
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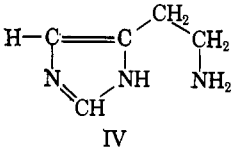

IV

(1) Taken in part from a thesis submitted by M. A. Doran to the faculty of Clark University in partial fulfillment of the requirements for the Master of Arts degree, June, 1958.

(2) Department of Chemistry, Illinois Institute of Technology, Chicago 16, Ill.

(3) I. Photaki, D. Schaufele, S. Fallab, and H. Erlenmeyer, *Helv. Chim. Acta*, **40**, 187 (1957).

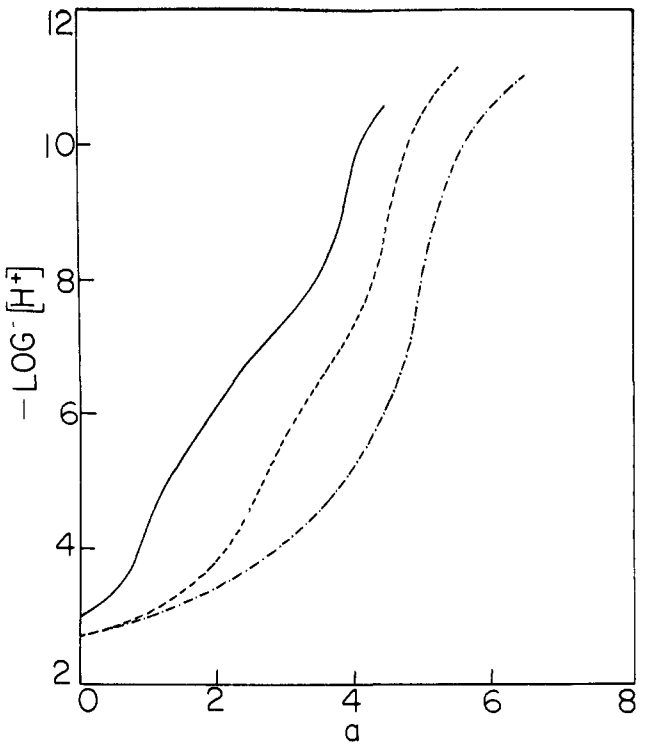


Fig. 1.—Potentiometric titration curves of histidylhistidine trihydrochloride, ———; histidylhistidine–Cu(II) 2:1, – – – –; histidylhistidine–Cu(II) 1:1, —·—·—; 25°, μ = 0.10 (KCl); a = moles of base added per mole of ligand present.

solution of this dipeptide and Cu(II) ions one more hydrogen ion is neutralized than in the titration of the ligand alone. They postulated formation of a Cu(II) complex which involves metal binding through the peptide linkage. No equilibrium constants for this reaction were given. Murphy[4] described a similar effect for the Cu(II) glycylglycine system and indicated that the dipeptide is a terdentate ligand in which the peptide group is involved. The Cu(II) stability constants of histamine have been investigated by Mickel and Andrews.[5] Similar measurements for the Cu(II) histidine system have been reported by Maley and Mellor[6] as well as by Li and co-workers.[7] Subsequent to the completion of the experimental work described in this paper,[8] there appeared in the literature a report by Martin and Edsall[9] which included some potentiometric work on the Cu(II) complex of histidylhistidine. Their work, carried out at high concentration ratios of peptide to metal ion, complements rather than duplicates the related portion of the present work.

Experimental

Apparatus and Procedure.—The experimental method consisted of the potentiometric titration of the ligand under investigation in the presence and absence of Cu(II) ions. Titrations of solutions containing 1:1 and 2:1 molar ratios of ligand to Cu(II) ion were made at 25 ± 0.05° at 0.1 ionic strength (KCl). The concentration of the ligand in the experimental solution was 2.5–3.0 × 10^{-3} M. The hydrogen ion concentration was measured with a Beckman Model GS pH meter with extension glass and calomel electrodes. The glass electrode was calibrated with the sodium acetate–acetic acid buffer system so as to correspond to hydrogen ion concentrations. Similar standardizations at high and low pH were effected by titrations with standard NaOH and HCl solutions. During all titrations a stream of presaturated CO_2-free nitrogen gas was passed through the reaction flask. Job's[10] method of continuous variations was used to determine the composition of the Cu(II) histidylhistidine complexes.

Materials.—Histamine dihydrochloride and *d,l*-histidine monohydrochloride were Eastman Organic White Label chemicals. Glycylglycine was obtained from the California Foundation for Biochemical Research. Histidylhistidine, obtained from the Mann Research Laboratories, came from the same batch (lot no. B1324) as that used by Martin and Edsall.[9] Standard hydrochloric acid was added to solutions of ligands to obtain the monohydrochloride form of glycylglycine and the trihydrochloride form of histidylhistidine. Concentrations of the ligand solutions were checked by potentiometric titration, while the standardization of the 0.0300 M Cu(II) stock solution was carried out by complexometric titration.

Calculations and Results

In the potentiometric curves of histamine dihydrochloride, there is an inflection at a = 1, while in those of histidine monohydrochloride and glycylglycine monohydrochloride, inflections at a values of 1 and 2 are observed. The acid dissociation constants of these three compounds were calculated algebraically in the usual way.

In the potentiometric curve of the trihydrochloride of histidylhistidine (Fig. 1), inflections occur at a = 1 and a = 4. A long sloping buffer region between these two inflections indicates the overlapping of the second, third, and fourth dissociations. The dissociation constant K_1 was evaluated by the usual relationship for a monobasic acid.

A modification of the procedure of Schwarzenbach and Ackermann[11] was used to obtain values for the remaining dissociation constants.

The potentiometric curves of histamine dihydrochloride and histidine monohydrochloride in the presence of Cu(II) ions indicate the stepwise formation of a 1:1 Cu(II) chelate, CuL, and a 2:1 Cu(II) chelate, CuL_2. The calculations of the Cu(II) chelate stability constants K_1 and K_2 of histamine and histidine, and the first formation constant K_1 of histidylhistidine, were carried out by an algebraic method and by a modification of Bjerrum's method in the manner described by Chaberek and Martell.[12]

Potentiometric equilibrium curves of equimolar ratios of glycyglycine hydrochloride and Cu(II) ion indicate that complex formation occurs with the neutralization of 3 equiv. of base, corresponding to the formation of a metal chelate, CuL*, in which a proton is displaced from the peptide linkage. The equilibria involved are

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

$$CuL^+ \rightleftarrows CuL^* + H^+ \qquad K_{CuL^*}{}^{H} = \frac{[CuL^+]}{[H^+][CuL^*]} \tag{2}$$

(4) C. B. Murphy, Dissertation, Clark University, 1952.
(5) B. L. Mickel and A. C. Andrews, *J. Am. Chem. Soc.*, **77**, 323, 5291 (1955).
(6) L. E. Maley and D. P. Mellor, *Nature*, **165**, 453 (1950).
(7) N. C. Li, E. Doody, and J. M. White, *J. Am. Chem. Soc.*, **79**, 5859 (1957).
(8) M. A. Doran, M.A. Thesis, Clark University, 1958.
(9) R. B. Martin and J. J. Edsall, *J. Am. Chem. Soc.*, **82**, 1107 (1960).
(10) P. Job, *Ann. Chim.*, **9**, 113 (1928).
(11) G. Schwarzenbach and H. Ackermann, *Helv. Chim. Acta*, **31**, 1029 (1948).
(12) S. Chaberek, Jr., and A. E. Martell, *J. Am. Chem. Soc.*, **74**, 5052 (1952).

The over-all reaction is given by

$$Cu^{2+} + L^- \rightleftarrows CuL^* + H^+$$

$$K_{CuL^*} = \frac{[H^+][CuL^*]}{[Cu^{2+}][L^-]} = \frac{K_{CuL}}{K_{CuL^*}{}^H} \quad (3)$$

For a first approximation of $K_{CuL^*}{}^H$ it is assumed that in the region $2 < a < 3$ only forms CuL^+ and CuL^* are present in appreciable amounts. If T_M and T_L represent total concentration of metal ion and metal chelate species, respectively, and a is the number of equivalents of base added per mole of ligand present, the following equation is obtained.

$$K_{CuL^*}{}^H = \frac{(3 - a)T_L - [H^+] + [OH^-]}{[H^+]((a - 2)T_L + [H^+] - [OH^-])} \quad (4)$$

If the presence of Cu^{2+} is not neglected, the relationship for $[CuL^+]$ is

$$[CuL^+] = \frac{\alpha(aT_L + [H^+] - [OH^-]) - \sigma T_L}{\alpha\theta - \psi\sigma} \quad (5)$$

where

$$\alpha = \frac{[H^+]^2}{K_1K_2} + \frac{[H^+]}{K_2} + 1$$

$$\sigma = \frac{[H^+]}{K_2} + 2$$

$$\theta = 2 + \frac{3}{[H^+]K_{CuL^*}{}^H}$$

$$\psi = 1 + \frac{1}{[H^+]K_{CuL^*}{}^H}$$

The first approximation of $K_{CuL^*}{}^H$ computed from eq. 4 was used to calculate $[CuL^+]$, $[L^-]$, and $[Cu^{2+}]$ from titration data between $a = 0$ and $a = 2$. These values were then used to calculate the first approximation of K_{CuL}. $[CuL^*]$ was then recalculated from the relationship

$$[CuL^*] = T_L - [HL] - [L^-] - [Cu^{2+}][L^-]K_{CuL} \quad (6)$$

The new value of $[CuL^*]$ thus obtained was used to recalculate $K_{CuL^*}{}^H$. In this second approximation, however, the presence of Cu^{2+} was not neglected. Thus a corrected value for $K_{CuL^*}{}^H$ was determined without neglecting overlapping of formation of CuL^+ and CuL^*. The two equilibrium constants K_{CuL} and $K_{CuL^*}{}^H$ were then recalculated by a series of successive approximations until the values became relatively constant.

The displacement of the peptide proton also occurs in the titration of an equimolar solution of histidylhistidine with Cu(II) ion. From the potentiometric curves (Fig. 1) it is seen that in the case of histidylhistidine the formation of CuL* does not overlap that of CuL. Therefore, the calculation of $K_{CuL^*}{}^H$ may be made from

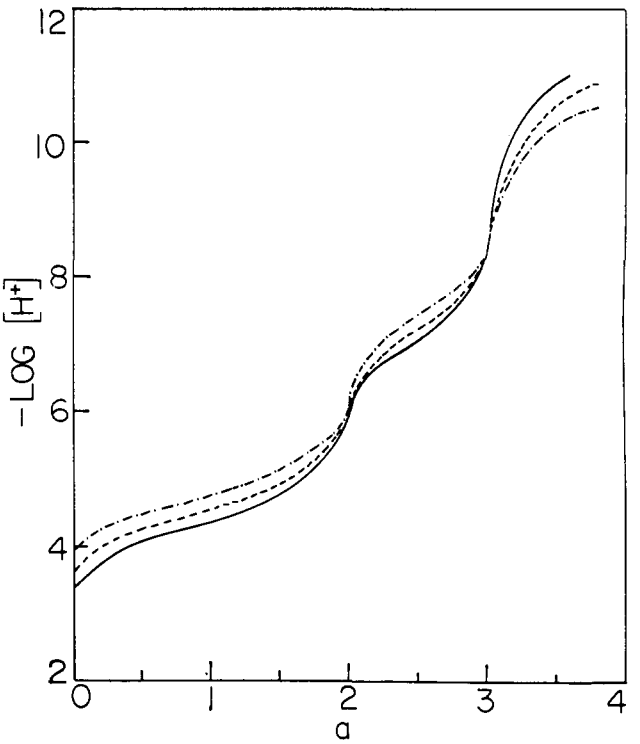


Fig. 2.—Potentiometric titration curves of 1:1 histamine dihydrochloride and Cu(II): 6.05×10^{-3} *M*, ———; 3.00×10^{-3} *M*, – – – –; 0.95×10^{-3} *M*, —·—·—; 25°, μ = 0.10 (KCl); a = moles of base added per mole of ligand present.

the equation

$$K_{CuL^*}{}^H = \frac{(5 - a)T_L - [H^+] + [OH^-]}{[H^+]((a - 4)T_L + [H^+] - [OH^-])} \quad (7)$$

where T_L and T_M have their previous meaning, and

$$aT_L + [H^+] - [OH^-] = 4[CuL] + 5[CuL^*] \quad (8)$$

Chelate stability constants calculated in accordance with the equations described above are listed in Table I.

In the formation of the monohydroxocopper(II) chelate of histamine, an increase in concentration results in a decrease in pH (Fig. 2), suggesting that a binuclear diolate chelate compound is formed. On this basis the equilibria may be defined by the following equations.

$$A_1 \rightleftarrows B_1 + H^+ \qquad K_{B_1} = \frac{[H^+][B_1]}{[A_1]} \quad (9)$$

$$A_1 \rightleftarrows B_2 + 2H^+ \qquad K_{B_2} = \frac{[H^+]^2[B_2]}{[A_1]} \quad (10)$$

$$2A_1 \rightleftarrows D + 2H^+ \qquad K_D = \frac{[H^+]^2[D]}{[A_1]^2} \quad (11)$$

where $A_1 = CuL^{2+}$, normal 1:1 chelate; $B_1 = CuL(OH)^+$; $B_2 = CuL(OH)_2$; $D = (CuL(OH))_2{}^{2+}$, the "dimer" of B_1. Under conditions such that no appreciable amount of dibasic species, $CuL(OH)_2$, is present, the usual stoichiometric relations for the species indicated in eq. 9–11 may be rearranged to give eq. 12.

TABLE I

ACID DISSOCIATION CONSTANTS AND CHELATE FORMATION CONSTANTS

Ligand	Formula of compound formed	Equilibrium quotient	log K[a]	Literature value
Histamine[h]	CuL^{2+}	$\frac{[CuL^{2+}]}{[Cu^{2+}][L]}$	9.48 ± 0.05	9.55[b]
	CuL_2	$\frac{[CuL_2^{2+}]}{[CuL^{2+}][L]}$	6.42 ± 0.05	6.48[b]
	$CuL(OH)^+$	$\frac{[CuL^{2+}]}{[H^+][CuL(OH)^+]}$	7.0 ± 0.0	...
	$(CuL(OH))_2^{2+}$	$\frac{[CuL]^2}{[H^+]^2[(CuL(OH))_2^{2+}]}$	11.8 ± 0.1	...
	$(CuL(OH))_2^{2+}$	$\frac{[(CuL(OH))_2^{2+}]}{[CuL(OH)^+]^2}$	2.2 ± 0.1	...
Histidylhistidine[i]	CuL^+	$\frac{[CuL^+]}{[Cu^{2+}][L^-]}$	12.0 ± 0.2	...
	CuL^*	$\frac{[CuL^+]}{[H^+][CuL^*]}$	6.21 ± 0.1	6.15[g]
Histidine[j]	CuL^+	$\frac{[CuL^+]}{[Cu^{2+}][L^-]}$	10.21 ± 0.05	10.60[e]
	CuL_2	$\frac{[CuL_2]}{[CuL^+][L^-]}$	8.32 ± 0.05	8.00[e]
Glycylglycine[k]	CuL^+	$\frac{[CuL^+]}{[Cu^{2+}][L^-]}$	6.52 ± 0.05	7.17[d] 5.88[g] 6.04[f]
	CuL^*	$\frac{[CuL^+]}{[H^+][CuL^*]}$	4.79 ± 0.05	5.38[d] 4.25[g]
	CuL^*	$\frac{[H^+][CuL^*]}{[Cu^{2+}][L^-]}$	1.73 ± 0.05	1.79[d] 1.63[g]

[a] This investigation, 25°, μ = 0.10 (KCl). [b] From Mickel and Andrews,[5] 25°, μ = 0.135 (KCl). [c] From Li, Doody, and White,[7] 25°, μ = 0.12–0.25. [d] From Murphy and Martell,[13] 30°, μ = 0.09 (KCl). [e] From Dobbie and Kermack,[18] 20°, ionic strength not specified. [f] From Monk,[14] 25°, zero ionic strength. [g] From Martin and Edsall,[9] 25°, ionic strength 0.16. [h–i] pK values for this research: [h] 6.02 ± 0.01, 9.70 ± 0.01. [i] 2.16 ± 0.05, 5.36 ± 0.01, 6.70 ± 0.01, 7.92 ± 0.01. [j] 5.98 ± 0.01, 9.08 ± 0.01. [k] 3.15 ± 0.02, 8.10 ± 0.01.

$$\frac{(a-2)T_L + [H^+] - [OH^-]}{[A_1]/[H^+]} = K_{B_1} + \frac{2K_D[A_1]}{[H^+]} = Y \quad (12)$$

A straight line obtained from a plot of Y *vs.* $[A_1]/[H^+]$ (Fig. 3) is evidence of the formation of a "dimer" of the normal 1:1 chelate. The slope of this line is equal to $2K_D$ and the intercept at $[A_1]/[H^+] = 0$ is equal to K_{B_1}. Values thus obtained are listed in Table I.

Spectrophotometric Measurements.—The histamine–Cu(II) complexes and glycylglycine–Cu(II) complexes exhibit absorption bands at 6100 and 6400 Å., respectively. A shift from 6100 to 6500 Å. of the absorption maximum of the histidylhistidine–Cu(II) band occurs when the pH is increased to 6. The optical density reaches a maximum at high pH values. In accordance with this shift in the absorption maximum, a color change at pH 5.6 from blue to purple is observed in the solution during the course of the potentiometric titration.

Figure 4 shows the variation of D_1, optical density, measured at the absorption maximum of 5600 Å., as the mole fraction of histidylhistidine is varied under conditions in which the total concentration of Cu(II) ion and ligand is constant. At pH 5.6 the fact that a maximum occurs at 0.5 mole fraction of histidylhistidine indicates the formation of a complex in which the molar ratio of ligand to metal is 1:1. The variation of D_1 at pH 8.6 is similar to that obtained for pH 5.6, but precipitation of the Cu(II) occurs in solutions which contain mole fractions of histidylhistidine less than 0.5.

Discussion

The value of 9.48 listed in Table I for log K of the Cu(II) histamine complex (V), which has 1 mole of ligand per gram-ion of metal, is somewhat lower than that of the analogous 1,3-diaminopropane complex (VI, log K = 9.77). This difference is probably due to the differences in basicities of histamine (pK_1 = 6.02, pK_2 = 9.70) and of the 1,3-diaminopropane (pK_1 = 8.98, pK_2 = 10.72.)[15] Since the affinities for hydrogen ions usually parallel affinities of ligands for

(13) C. B. Murphy and A. E. Martell, *J. Biol. Chem.*, **226**, 37 (1957).
(14) C. B. Monk, *Trans. Faraday Soc.*, **47**, 292, 297 (1951).
(15) H. Irving, R. J. P. Williams, D. J. Ferrett, and A. E. Williams, *J. Chem. Soc.*, 3494 (1954).

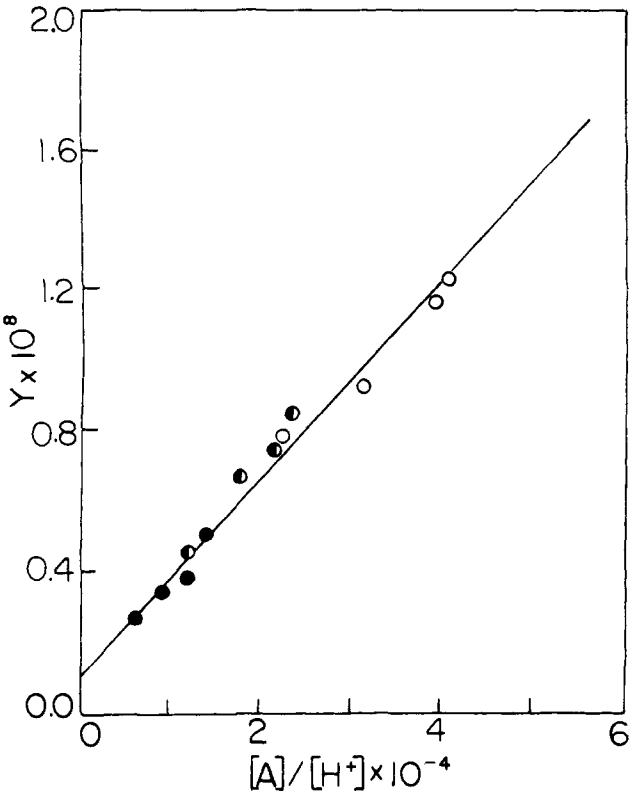


Fig. 3.—Graph of $\{(a - 2)T_L + [H^+] - [OH^-]/([A_1]/[H^+])\} = Y$, plotted *vs.* $[A_1]/[H^+]$, $-\log [H^+]$ between 6.8 and 7.8: histamine–Cu(II) 1:1, ●, 0.95×10^{-3} *M*; ◐, 3.00×10^{-3} *M*; ○, 6.05×10^{-3} *M*.

metal ions, the observed difference in stabilities of these analogous chelate compounds is probably considerably less than would have been predicted on the basis of the relative basicities of the ligands. This points to some additional stabilization of the histamine chelate, which may be due in part to the rigidity of the imidazole ring, and to possible π-bonding between the imidazole ring and the metal ion.

Since the proposed structure of the 1:1 histidine–Cu(II) chelate compound (VII) is similar to the analogous histamine chelate (V) and since the basicity of histidine is somewhat lower than that of histamine, one would expect a lower stability constant for the histidine–Cu(II) chelate. The fact that the reverse is true may be taken as evidence that the carboxylate group of histidine is also involved in metal binding, in spite of the strain involved in such an interaction.

This influence of the carboxyl group on the stability also carries through to the 2:1 chelates (VIII and IX), as may be noted from the corresponding values of log K_{CuL_2} in Table I. In the case of the 2:1 histidine–Cu(II) chelate it is interesting to note that involvement of the carboxylate binding seems to require the formation of a tetragonal arrangement of donor groups with carboxylate groups above and below the plane which contains only the basic nitrogen atoms and the metal ion.

The reactions of the two dipeptides, glycylglycine and histidylhistidine, are similar in that two types of 1:1 complexes are postulated for both ligands. In the normal 1:1 glycylglycine–Cu(II) complex, coordination probably takes place through the terminal amino and carboxyl groups of the ligand as is indicated

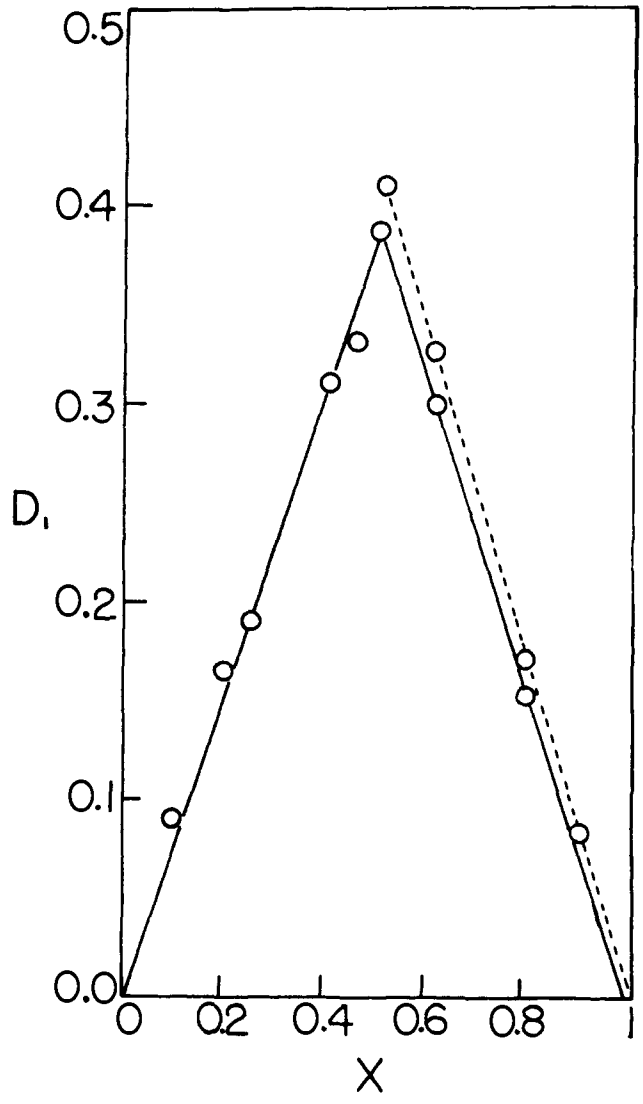
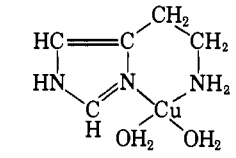


Fig. 4.—Continuous variation of histidylhistidine–Cu(II): *X*, mole fraction of histidylhistidine (total concentration of ligand and Cu(II), 1.8×10^{-2} *M*); D_1, optical density at 5600 Å.; pH 5.6, ———; pH 8.6, - - - -.

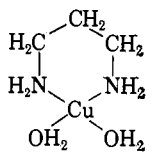
by formula X. In the case of histidylhistidine, metal binding probably involves the nitrogens of the two imidazole rings and at least one of the other possible metal coordination sites, probably the amino nitrogen or possibly the carboxylate group (XI). With respect to both complexes, potentiometric data preclude the involvement of the peptide linkage except in some weakly associated manner.

A comparison of the stability constants listed in Table I for all the normal 1:1 chelate compounds, which involve no displacement of the peptide hydrogen atom, shows that histidylhistidine has the greatest affinity for the Cu(II) ion. This observation is in accordance with the fact that formula XI, which is suggested as the structure of the metal chelate compound formed, contains a larger number of coordinate linkages than do V, VII, and X.

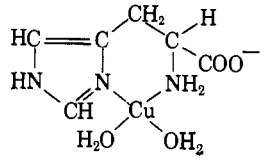
A comparison of the dissociation constants of the normal Cu(II)–peptide chelate compounds (CuL^+ of Table I) shows that the tendency of the hydrogen ion to dissociate from the peptide linkage is greater in the case of the Cu(II) chelate of glycylglycine (X) than for



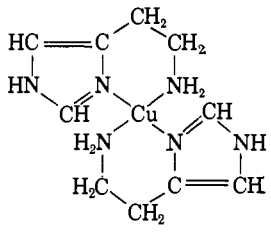
V



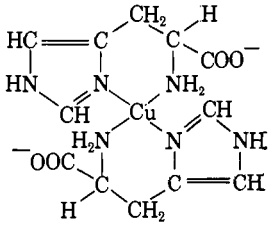
VI



VII



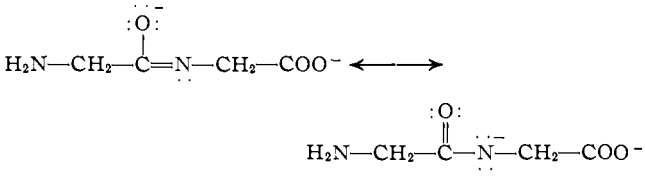
VIII



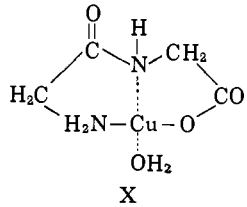
IX

that of histidylhistidine (XI). This difference is reasonable in view of the fact that the normal 1:1 glycylglycine–Cu(II) complex has relatively low stability (log K_{CuL} = 6.52), while in the histidylhistidine complex, the Cu(II) ion is coordinated with at least three basic groups (log K_{CuL} = 12.0). Hence the polarizing effect of Cu(II) on the peptide linkage would be expected to be much weaker in the latter chelate compound. Probable structures of the chelate compounds of glycylglycine and histidylhistidine in which the peptide linkage becomes a negative, strongly coordinated donor group are illustrated by XII and XIII.

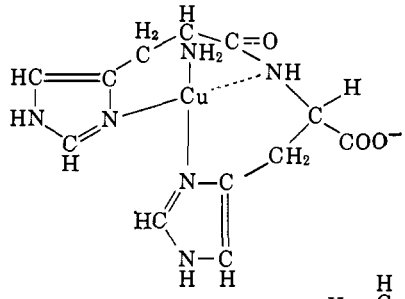
It should be noted that structures XIII and XIV involve coordination of the nitrogen and not the oxygen of the peptide linkage. There has been some disagreement as to which of these atoms enter into coordination with metal ions. Manyak, *et al.*,[16] and Dobbie and Kermack[17] postulate the involvement of the peptide nitrogen in the glycylglycine–Cu(II) complex. Rabin[18] proposed the peptide oxygen as a metal coordination site in the Co(II) and Mn(II) complexes. The latter structure is also favored by Li, *et al.*[7] A molecular model of Cu(II) glycylglycine, based on the intermediate structure



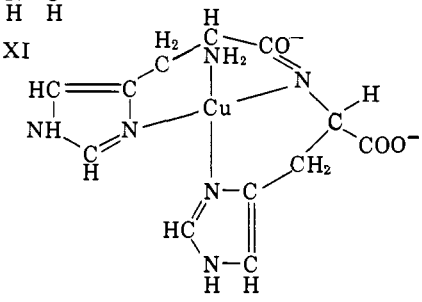
was made. From this model, it can be seen that coordination of the terminal amino group and of either the peptide nitrogen or oxygen is sterically possible.



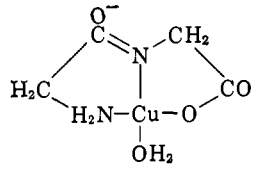
X



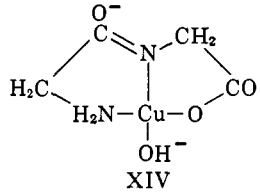
XI



XII



XIII



XIV

If the peptide nitrogen is used as a binding site the terminal carboxylate group becomes a possible metal coordination site. This is not the case with peptide oxygen linkage. Involvement of the acetate group, whether by direct coordination or by a steric screening effect, is in agreement with observations made on the basis of potentiometric data which suggest that glycylglycine is effectively terdentate rather than bidentate with respect to Cu(II) ion.

Since Cu(II) is coordinated to three or more binding groups when present in the form of the Cu(II) peptide, it might be expected that the formation of a chelate having a ligand to Cu(II) ratio of 2:1 would not occur. This fact is borne out by potentiometric data for the 2:1 ligand–metal systems involving both glycylglycine and histidylhistidine, since the 2:1 titration curves of Fig. 1, for example, may be constructed by addition of the abscissas of potentiometric curves of the 1:1 metal chelate system to those of the free ligand. The same conclusion has also been made on the basis of the continuous variations study of histidylhistidine. A similar observation has also been made for the Cu(II)–carnosine chelate by Dobbie and Kermack,[17] and for the Cu(II)–glycylglycine system by Murphy and Martell.[13] These results are not inconsistent with the detection of a 2:1 glycylglycine–Cu(II) chelate by Dobbie and Kermack[17] at higher concentrations and ratios of ligand to metal ion.

The values of the constants of the hydrolyzed form of the 1:1 histamine–Cu(II) chelate (log ($[CuL^{2+}]/[CuL$-

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$OH^+][H^+]) = 7.0$) and of the dimeric form (log ($[(CuL-[OH])_2^{2+}]/[CuL(OH)^+]^2$) = 2.2) fall in the range of values reported by Gustafson and Martell[19] for a series of related bidentate ligands.

A similar hydrolysis reaction appears to take place in glycylglycine–Cu(II) titrations at high pH values. The postulated structure (XIII) has one molecule of water coordinated to the Cu(II) ion. A reaction resulting in the formation of a monohydroxo chelate is therefore possible. Dimerization of the monohydroxo chelate probably does not occur because of the negative charge of the $CuL(OH)^-$ (XIV), in accordance with the generalizations discussed previously for dimerization of Cu(II) chelates.[20]

The structure suggested for the Cu(II)–histidylhistidine peptide chelate contains no water molecule coordinated to Cu(II). The absence of an appreciable tendency toward hydrolysis is confirmed by the steep inflection of the titration curve of Fig. 1 at $a = 5$ followed by a high pH buffer region at pH values of 10 or above.

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Interaction of Alkyllithium Compounds with Base. Complex Formation between Ethyllithium and Triethylamine in Benzene

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Solutions of ethyllithium with varying quantities of added triethylamine in benzene have been studied using dielectric constant and freezing point lowering measurements. The results demonstrate that interaction between the two solute species does occur and provide evidence that a number of related equilibria are involved. It is proposed that, at low ratios of base to alkyllithium, coordination of base occurs to the intact alkyllithium hexamer. As the base:hexamer ratio increases, the hexamer dissociates into coordinated dimer. The species $(RLi)_2(base)_2$ is formed initially, but may be further solvated at higher base concentrations. It is further proposed that the equilibria postulated also characterize alkyllithium solutions in other bases, notably ether. Infrared, n.m.r., and kinetic data are consistent with the proposed equilibria.

The structures of alkyllithium compounds in the vapor[2,3] and solid states,[4] and in hydrocarbon solutions,[3,5] are reasonably well established at this time. There remains, however, an uncertainty as to the form in which the compounds exist in basic, solvating media. The effect of small quantities of a Lewis base on the reactivity of alkyllithium compounds in hydrocarbon solutions,[6-8] particularly in connection with anionic polymerization reactions,[9-13] is a related problem which has attracted considerable interest in recent years.

It is well known that the alkyllithium compounds are much more reactive in ether than in hydrocarbon media.[14] Since this reactivity extends to reaction with the solvent itself, attempts to determine the degree of association in ether by ebulliometry have not been notably successful.[15,16] Further, the danger of contamination from oxygen or water vapor is enhanced in this type of experiment.

Infrared spectra of alkyllithium compounds in basic solvents have been reported.[17-19] The behavior of the 500 cm.$^{-1}$ band, which exhibits a large ^6Li–^7Li isotope shift, is quite interesting.[17] It seems very likely that this band is due largely to carbon–lithium stretching–bending in the polymeric species; it should therefore be quite sensitive to the state of the alkyllithium compound. The band appears at 530 cm.$^{-1}$ in hydrocarbon solvent and is shifted to lower frequencies in ether (492 cm.$^{-1}$), benzene plus ether (511 cm.$^{-1}$), and benzene plus triethylamine (520 cm.$^{-1}$). The fact that the bands do shift appreciably demonstrates that interaction with base does occur. On the other hand, the shifts are smaller than one would expect if the interaction led to complete disruption of the polymeric alkyllithium species. It is possible, even probable, that in the presence of base the polymer is partially disrupted, with some form of multicenter bond persisting in the resulting complex. But a complete disruption of the polymer to give solvated monomer complexes should result in a drastic shift to lower frequency of the 500 cm.$^{-1}$ band.[20] The infrared data therefore indicate some self-association of alkyllithium compounds in basic solutions.

Indirect evidence relating to interaction of alkyllithium compounds with bases has also been obtained from kinetic studies.[6,9,13]

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(20) In the limiting case of a solvated ion pair, the carbon–lithium stretching force constant should be very low, its magnitude determined by the degree of "intimateness" of the ion pair.