Copper(II)–Histidine Complexes

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Abstract: A thermodynamic study of the interaction of copper(II) in aqueous solution with histidine and a series of ligands structurally related to histidine is reported. The ligands studied are histidine, histidine methyl ester, histamine, and imidazolepropionic acid. Calorimetric ΔH and potentiometric ΔG values were determined at 25° and at an ionic strength of 0.16 (KNO_3). The thermodynamic values obtained for the bonding of anionic histidine to copper(II) are similar to those for histamine and histidine methyl ester, which suggests amino–imidazole chelation. Participation of the carboxyl group is uncertain. The magnitude of the ΔH value for the coordination of the monoprotonated form of the histidine molecule indicates that the carboxyl group of histidine is protonated. The amino and imidazole groups are again involved in bonding. Thermodynamic values for the protonation reactions of the ligands are also reported.

The reaction of copper(II) with histidine in aqueous solution is complicated by the fact that the square-planar tendency of copper(II) allows only two of the three available bonding sites of histidine to be bound to the metal ion in the plane, while the third site may interact weakly, if at all, in the axial positions. This permits the histidine molecule to bond as both the unprotonated and the monoprotonated ligand and requires complicated equilibrium expressions to describe the complex formation.

A thermodynamic approach to this problem is described in the present work. It was hoped that thermodynamic results coupled with the available literature could be used to describe the bonding in the copper(II)–histidine system. To aid in this study, ligands closely related to histidine with respect to functional grouping, but containing only two potential bonding sites in common with histidine, were investigated. Histamine, histidine methyl ester, and β-(4-imidazole)propionic acid were chosen since the relative positions of potential bond sites are identical with those of the parent compound histidine (Figure 1). A series of simple amino acids, related to alanine, was studied earlier[1] and provides data for amino and carboxyl coordination.

The difficulty in describing the copper(II)–histidine system by conventional potentiometric techniques was first noted by Albert,[2] although formation constants were reported earlier by Maley and Mellor.[3] Consistent values for the first formation constant could apparently be obtained; however, progressively smaller values of the second constant were calculated as the reaction proceeded. Similar complications were later reported by other workers.[4–12] It was agreed, however, that both amino and imidazole groups must be involved, since the approximate stability constants were similar to that of histamine and several orders of magnitude higher than the values reported for chelates containing a carboxyl and either an amino or imidazole group.

The possibility that the monoprotonated histidine was acting as a ligand was suggested by Datta, *et al.*[5] Leberman and Rabin[6] later calculated equilibrium constants for these reactions by a combination of graphical and approximation methods. Perrin,[7] using an iterative method, and Perrin and Sharma,[12] using computer techniques, have obtained consistent results.

The first quantitative study of the formation of the copper(II)–histamine complexes was made by Albert.[2] Later Mikel and Andrews[13] reported formation constants for the mono and bis complexes and found no evidence for higher order complexes. More recently[6,8,9,14,15] other studies have been made with similar results.

Stability constants for the reaction of histidine methyl ester with copper(II) were published by Andrews and Zebolsky,[11] Li, *et al.*,[4] and Conley and Martin.[16]

The only literature values available for β-(4-imidazole)propionic acid were supplied by Chakravorty and Cotton.[9] They attempted a procedure similar to that of the present study to explain the bonding in histidine utilizing only stability constants.

Enthalpy data have been provided by temperature variation methods for histamine,[14,15] histidine,[11,17,18] and histidine methyl ester.[11] The overall heat of formation of the bis(histidinato)copper(II) complex has been determined calorimetrically.[19]

Experimental values for the protonation constants for these systems are numerous and can be found in standard compilations.[20,21]

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Experimental Section

Materials. Carbonate-free sodium hydroxide was obtained from Bio-Rad Laboratories and the nitric acid solutions were prepared from Baker Analyzed Reagent grade acid. These solutions were standardized by conventional methods. All inorganic salts were of reagent grade. Copper nitrate solutions were analyzed by electrodeposition. All solutions were prepared with an ionic strength of 0.16 with deionized distilled water. Potassium nitrate was the added electrolyte in all cases.

L-Histidine (free base), histamine, and L-histidine methyl ester (dihydrochlorides) were obtained as solids from Nutritional Biochemicals Corp. They were used without further purification after drying at 110° for 1 hr. Imidazolepropionic acid was prepared by the method of Windaus and Vogt[22] and was obtained as the hydrochloride monohydrate, mp 78–79° (lit. mp 80°).

Anal. Calcd for $C_6H_8N_2O_2 \cdot HCl \cdot H_2O$: C, 40.8; H, 5.1; N, 15.9, equiv wt, 192.6. Found: C, 41.8; H, 5.4; N, 16.3, equiv wt, 192.8.

Potentiometric Titrations. The titrations were carried out in a manner similar to that previously described.[1] Exceptions were additional stability constant titrations for histidine, histamine, and histidine methyl ester. Because of the possibility of mixed complex formation (ligands bonding in a protonated form) titrations were performed by adding ligand solution to a copper(II) solution. In this way the pH of the solution could be regulated since uncomplexed ligand would serve to buffer the reaction solution. By varying the degree of protonation of the titrant the complexation reaction could be studied over a fairly wide range of pH values. Ligand–metal mole ratios of 2–5 to 1 were used. The larger ratios were required as the pH was lowered. Temperature was maintained at 25.0 ± 0.1° by circulating water from a constant temperature bath.

Calorimetric Titrations. The calorimeter used for the enthalpy titrations has been described.[1] The method used for determining the heats of protonation has also been described.[1] Enthalpy titrations for the complexation reactions of histidine, histidine methyl ester, histamine, and imidazolepropionic acid with copper(II) were performed by adding the partially protonated form of the ligands to the metal ion solution. The pH of the solutions was monitored so that the concentration of all species could readily be calculated and appropriate corrections for the heats of protonation could be made. Ligand–metal mole ratios of 2–5 to 1 were again used depending on the pH of the solution. All calorimetric titrations were performed at 25.00 ± 0.01°.

Results

Potentiometric Titrations. Acid Dissociation Constants. Since the successive protonation constants for the ligands histidine, histamine, and imidazolepropionic acid are well separated, conventional methods were used to calculate the constants using the measured pH. The two protonation constants for histidine methyl ester, however, are too close to neglect overlap in the buffer regions and the following linear equation was used to calculate the constants.

$$\frac{[H^+]^2(\bar{n} - 2)}{\bar{n}} = K_1\frac{[H^+](1 - \bar{n})}{\bar{n}} - K_1K_2 \qquad (1)$$

Values for $\bar{n}$ can be calculated from experimentally measured quantities; $\bar{n}$ is defined as the average number of protons bound per ligand. K_1 and K_2 refer to the first and second acid dissociations, respectively, of histidine methyl ester (charges are omitted for clarity).

$$H_2L \rightleftharpoons HL + H^+ \qquad K_1 = \frac{[HL][H^+]}{[H_2L]} \qquad (2)$$

$$HL \rightleftharpoons L + H^+ \qquad K_2 = \frac{[L][H^+]}{[HL]} \qquad (3)$$

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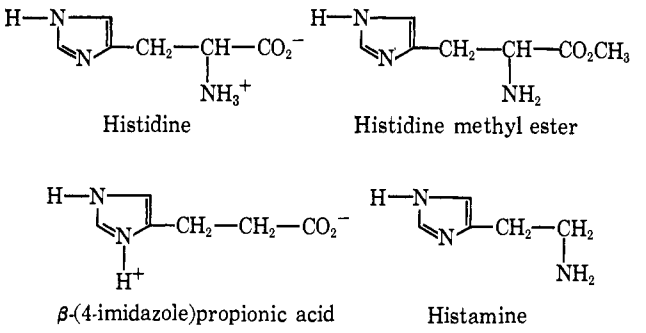

Figure 1. Derivatives of histidine.

A plot of $[H^+]^2(\bar{n} - 2)/\bar{n}$ *vs.* $[H^+](1 - \bar{n})/\bar{n}$ yields a straight line of slope K_1 and intercept $-K_1K_2$. The data were fitted to eq 1 by a linear least-squares procedure. The experimental protonation values for all the ligands are listed in Table I, where comparison is made with appropriate literature values.

Table I. Acid Dissociation Constants[a]

	p*K* (amino)	p*K* (imidazole)	p*K* (carboxyl)	Ionic strength
Histidine	9.21[b]	6.17[b]	2.02[b]	0.16
	9.17[c]	6.08[c]	1.77[c]	0.20
	9.17[d]	6.12[d]	1.96[d]	0.25
	9.08[e]	5.98[e]		0.10
	9.20[f]	6.17[f]		0.15
Histidine methyl ester	7.37[b]	5.39[b]		0.16
	7.34[d]	5.38[d]		0.25
	7.33[f]	5.38[f]		0.15
	7.30[g]	5.35[g]		0.16
Histamine	9.97[b]	6.25[b]		0.16
	9.87[c]	6.20[c]		0.20
	9.70[e]	6.02[e]		0.10
	9.88[h]	6.13[h]		0.14
Imidazole-propionic acid		7.57[b]	3.96[b]	0.16
		7.56[c]	3.95[c]	0.20

[a] All values reported are at 25.0°. [b] Present study. [c] Reference 9. [d] Reference 11. [e] Reference 8. [f] Reference 4. [g] Reference 16. [h] Reference 14.

Stability Constants. The method of calculation previously described[1] was used to obtain values for histamine, histidine methyl ester, and imidazolepropionic acid. Results for these systems are presented in Table II, where comparison is made with selected literature values. The copper(II)–histidine system, however, is complicated by the fact that the histidine molecule can coordinate to copper ion in solution with the completely deprotonated or a monoprotonated form of the ligand or both. The following equilibria are necessary to describe the formation of all possible mono- and bis(histidinato)copper(II) complexes. The charges are omitted for clarity. L represents the completely deprotonated ligand and HL a monoprotonated form.

$$Cu + L \rightleftharpoons CuL \qquad K_1 = \frac{[CuL]}{[Cu][L]} \qquad (4)$$

$$CuL + L \rightleftharpoons CuL_2 \qquad K_2 = \frac{[CuL_2]}{[CuL][L]} \qquad (5)$$

$$Cu + HL \rightleftharpoons CuHL \qquad K_1' = \frac{[CuHL]}{[Cu][HL]} \qquad (6)$$

$$CuHL + HL \rightleftharpoons Cu(HL)_2 \qquad K_2' = \frac{[Cu(HL)_2]}{[CuHL][HL]} \qquad (7)$$

$$CuHL + L \rightleftharpoons Cu(HL)L \qquad K_2'' = \frac{[Cu(HL)L]}{[CuHL][L]} \qquad (8)$$

Table II. Stability Constants for the Histidine Derivatives[a]

	Log K_1	Log K_2	Ionic strength
Histamine	9.56[b]	6.37[b]	0.16
	9.43[c]	6.43[c]	0.20
	9.55[d]	6.48[d]	0.14
	9.48[e]	6.42[e]	0.10
Histidine methyl ester	8.46[b]	5.92[b]	0.16
	8.48[f]	5.90[f]	0.16
	9.10[g]	5.70[g]	0.15
	8.32[h]	5.82[h]	0.25
Imidazole-propionic acid	4.46[b]	4.03[b]	0.16
	4.56[c]	3.89[c]	0.20

[a] All values reported are at 25.0°. [b] Present study. [c] Reference 9. [d] Reference 14. [e] Reference 8. [f] Reference 16. [g] Reference 4. [h] Reference 11.

Since the five independent equilibrium constants cannot be calculated analytically from the experimental data, a least-squares computer program, similar in principle to that described by Unwin, *et al.*,[23] for the simpler two-equilibrium problem, was written to find the set of constants which best fit the data to a specified theoretical curve. The average number of ligands bound per metal ion, $\bar{n}$, was chosen as the function to be approximated. If all possible species indicated in eq 4–8 are assumed to be formed, $\bar{n}$ can be expressed by the following equation.

$$\bar{n} = \frac{[\mathrm{CuL}] + 2[\mathrm{CuL_2}] + [\mathrm{CuHL}] + 2[\mathrm{Cu(HL)_2}] + 2[\mathrm{Cu(HL)L}]}{[\mathrm{Cu}] + [\mathrm{CuL}] + [\mathrm{CuL_2}] + [\mathrm{CuHL}] + [\mathrm{Cu(HL)_2}] + [\mathrm{Cu(HL)L}]} \quad (9)$$

Substituting eq 4–8 into eq 9 and canceling like terms gives

$$\bar{n} = \frac{K_1[\mathrm{L}] + 2K_1K_2[\mathrm{L}]^2 + K_1'[\mathrm{HL}] + 2K_1'K_2'[\mathrm{HL}]^2 + 2K_1'K_2''[\mathrm{HL}][\mathrm{L}]}{1 + K_1[\mathrm{L}] + K_1K_2[\mathrm{L}]^2 + K_1'[\mathrm{HL}] + K_1'K_2'[\mathrm{HL}]^2 + K_1'K_2''[\mathrm{HL}][\mathrm{L}]} \quad (10)$$

Given $[([\mathrm{L}]_i, [\mathrm{H}^+]_i, \bar{n}_i)$: $i = 1, 2, \ldots, N]$, where N is the number of data points, the problem is to determine the set of equilibrium constants (K_1, K_2, K_1', K_2', K_2'') which minimize

$$F(K_1, K_2, K_1', K_2', K_2'') = \sum_{i=1}^{N}[\bar{n}_i - f([\mathrm{L}]_i, [\mathrm{H}^+]_i; K_1, K_2, K_1', K_2', K_2'')]^2 \quad (11)$$

where $f([\mathrm{L}]_i, [\mathrm{H}^+]_i; K_1, K_2, K_1', K_2', K_2'')$ is the right-hand side of eq 10.

The hydrogen ion concentration was measured potentiometrically. The free ligand concentration was calculated from the proton balance equations. C_A, C_B, and C_H are defined as total concentrations of acid, base, and hydrogen ion, respectively.

$$C_H = C_A - C_B \quad (12)$$

and

$$C_H = [\mathrm{H}^+] + [\mathrm{HL}] + 2[\mathrm{H_2L}] + 3[\mathrm{H_3L}] + [\mathrm{CuHL}] + [\mathrm{Cu(HL)L}] + 2[\mathrm{Cu(HL)_2}] - [\mathrm{OH}^-] \quad (13)$$

Equating eq 12 and 13 and substituting the proper protonation and formation constants, the following equation is obtained upon rearrangement.

$$[\mathrm{L}] = \frac{C_A - C_B - [\mathrm{H}^+] + [\mathrm{OH}^-]}{\{[\mathrm{H}^+]/K_{a_3} + 2[\mathrm{H}^+]^2/K_{a_2}K_{a_3} + 3[\mathrm{H}^+]^3/K_{a_1}K_{a_2}K_{a_3} + K_1'[\mathrm{H}^+][\mathrm{Cu}]/K_{a_3} + 2K_1'K_2'[\mathrm{H}^+]^2[\mathrm{L}][\mathrm{Cu}]/(K_{a_3})^2 + K_1'K_2''[\mathrm{H}^+][\mathrm{Cu}][\mathrm{L}]/K_{a_3}\}} \quad (14)$$

K_{a_1}, K_{a_2}, and K_{a_3} are the acid dissociation constants which apply to removal of a proton from the carboxyl, imidazole, and amino groups, respectively. The uncomplexed metal ion concentrations must be known in order to calculate the free ligand concentration from eq 14. This can be calculated from the metal balance.

$$C_M = [\mathrm{Cu}] + [\mathrm{CuL}] + [\mathrm{CuHL}] + [\mathrm{CuL_2}] + [\mathrm{Cu(HL)_2}] + [\mathrm{Cu(HL)L}] \quad (15)$$

Substituting eq 4–8 into (15) and solving for free metal concentration, eq 16 is derived. Since eq 14 and 16

$$[\mathrm{Cu}] = \frac{C_M}{1 + K_1[\mathrm{L}] + K_1'[\mathrm{HL}] + K_1K_2[\mathrm{L}]^2 + K_1'K_2'[\mathrm{HL}]^2 + K_1'K_2''[\mathrm{HL}][\mathrm{L}]} \quad (16)$$

contain terms in both the free metal and free ligand concentration, an iterative method must be used. The procedure was to first estimate the free ligand concentration assuming no protonated complexes and use this value to estimate the metal concentration and the free ligand concentration from eq 16 and 14, respectively. Iteration of [Cu], [L], and $\bar{n}$ were performed until self-consistant values were obtained. $\bar{n}$ was calculated from the ligand balance by

$$\bar{n} = \frac{C_L - [\mathrm{L}](1 + [\mathrm{H}^+]/K_{a_3} + [\mathrm{H}^+]^2/K_{a_2}K_{a_3} + [\mathrm{H}^+]^3/K_{a_1}K_{a_2}K_{a_3})}{C_M} \quad (17)$$

Estimated values of the formation constants were used for the first set of iterations.

After consistent values of the independent variables were obtained, the least-squares problem defined by eq 11 was solved. This was accomplished by varying each of the parameters (K_1, K_2, K_1', K_2', K_2'') in turn over a specified interval and calculating the squares of the deviation of the theoretical from the calculated $\bar{n}$ values. The value of the constant which gave the smallest deviation was then stored and used for the calculation of the next constant. This was allowed to proceed until all "best" values for the stability constants were obtained. At this point, the free ligand concentration for each of the data points was again calculated by the iterative procedure described above except that the starting values for [L] were the values obtained during the preceding iteration and the formation constants used in eq 14 and 16 were the values calculated from the least-squares treatment. The complete process was repeated, each time decreasing the interval over which the constants can vary until the required convergence was attained. In practice the iterations were allowed to continue until the maximum deviation at any point from the theoretical curve was 0.01 $\bar{n}$ unit.

(23) E. A. Unwin, R. G. Beimer, and Q. Fernando, *Anal. Chim. Acta*, 39, 95 (1967).

In general, the success of the least-squares fitting depends on the initial selection of reasonable values of the stability constants. Fortunately, these can usually be obtained by graphical methods, or by comparison with similar chemical systems. The method has the advantage that convergence to "false" minimums cannot occur, since the actual minimum is chosen from the set of $F(K_1, K_2, K_1', K_2', K_2'')$ values. The results obtained for the copper(II)–histidine system are presented in Table III along with other reported data. A consistent

Table III. Stability Constants for Histidine

Log K_1	Log K_2	Log K_1'	Log K_2''
10.01[a] (±0.07)	8.01[a] (±0.02)	4.37[a] (±0.13)	10.13[a] (±0.09)
10.37[b]	[c]	5.02[b]	[c]
10.35[d]	8.35[d]	5.07[d]	10.06[d]
9.79[e]	7.62[e]	5.11[e]	9.02[e]

[a] Present study; errors represent standard deviations of five independent titrations. [b] Reference 6, 25°, μ = 0.01. [c] Values were not calculated. [d] Reference 7, 20°, μ not given. [e] Reference 12, 40°, μ = 0.16.

value of K_2' could not be calculated. Calculations using an estimated value of K_2' showed that the concentration of the species $Cu(HL)_2^{2+}$ would be negligibly small under the present experimental conditions.

Calorimetric Titrations. Heats of Deprotonation. The heat of protonation for the carboxyl group of histidine was determined by adding a nitric acid solution to the partially deprotonated histidine. Since the acid constant for carboxyl deprotonation is relatively large, corrections were applied for added titrant not used for the protonation reaction.

A linear least-squares method was used to treat the remaining two deprotonations of histidine as well as the other dibasic ligands. The two heats of protonation can be determined simultaneously by fitting the data to the equation derived below. ΔH_1 and ΔH_2 refer to dissociating reactions 2 and 3, respectively. ΔH_1 represents the imidazole group for all ligands, except imidazolepropionic acid (carboxyl group), with ΔH_2 representing the heat of deprotonation of the most basic functional group. If $\overline{\Delta H}$ is defined as the average heat per mole of ligand protonated, then

$$\overline{\Delta H} = \frac{\Delta H_1[HL] + (\Delta H_1 + \Delta H_2)[H_2L]}{[HL] + 2[H_2L]} \quad (18)$$

Substituting eq 2 and 3 into 18 and canceling like terms, the following equation is obtained.

$$\overline{\Delta H} = \frac{\Delta H_1 + (\Delta H_1 + \Delta H_2)[H^+]/K_1}{1 + 2[H^+]/K_1} \quad (19)$$

This can be arranged into the form of a linear equation

$$\overline{\Delta H}(1 + \theta) = \Delta H_2\theta + \Delta H_1 \quad (20)$$

where

$$\theta = \frac{[H^+]/K_1}{1 + [H^+]/K_1}$$

Therefore a plot of $\overline{\Delta H}(1 + \theta)$ *vs.* θ yields a straight line of slope ΔH_2 and intercept ΔH_1.

Table IV. Heats of Deprotonation

	ΔH[a] (amino)	ΔH (imidazole)	ΔH(carboxyl)
Histidine	10.43[b] (C)[c]	7.01[b] (C)	0.72[b] (C)
	9.40[d] (T)[e]	7.20[d] (T)	
Histidine methyl ester	9.97[b] (C)	7.57[b] (C)	
Histamine	12.55[b] (C)	7.42[b] (C)	
	13.40[f] (T)	10.10[f] (T)	
	9.00[g] (T)	7.80[g] (T)	
Imidazole-propionic acid		8.82[b] (C)	−0.01[b] (C)

[a] In kcal/mol. All enthalpy values are for 25°. [b] Present study. [c] Calorimetrically. [d] Reference 17. [e] Temperature variation. [f] Reference 16. [g] Reference 14.

The results for all the systems are summarized in Table IV. The experimental accuracy is estimated to be ±0.5% from the standard error of the slope and intercept of the least-squares treatment. Comparison is made with available literature values.

Heats of Complexation. The heats of formation of the mono and bis copper(II) complexes of histamine, histidine methyl ester, and imidazolepropionic acid were calculated from the experimental data as previously described.[1] Corrections were made for competing protonation–deprotonation reactions of the ligands using the calculated concentrations of the various species and the previously determined heats of deprotonation. The results for these systems are given in Table V where other available literature values are listed. The experimental accuracy is again estimated to be ±0.5%.

Table V. Heats of Complexation of the Histidine Derivatives

	$-\Delta H_1$[a]	$-\Delta H_2$
Histamine	13.48[b] (C)[c]	10.84[b] (C)
	7.0[d] (T)[e]	7.0[d] (T)
	15.5[f] (T)	11.6[f] (T)
Histidine methyl ester	11.04[b] (C)	8.16[b] (C)
	18.3[g] (T)	16.0[g] (T)
Imidazole-propionic acid	3.65[b] (C)	5.15[b] (C)

[a] In kcal/mol. All enthalpy values are for 25°. [b] Present study. [c] Calorimetrically. [d] Reference 14. [e] Temperature variation. [f] Reference 16. [g] Reference 11.

The enthalpy values for the formation of the five species indicated in eq 4–8 were calculated by a least-squares procedure similar to that used for the calculation of the formation constants. The function to be approximated, however, is $\overline{\Delta H}$ the average heat per mole of ligand bound. ΔH_1, ΔH_2, $\Delta H_1'$, $\Delta H_2'$, and $\Delta H_2''$ refer to reactions 4–8, respectively. $\overline{\Delta H}$ can be expressed by eq 21, assuming that only the five species above are formed. Substituting the expressions for the

$$\overline{\Delta H} = \frac{\begin{array}{l}\Delta H_1[CuL] + (\Delta H_1 + \Delta H_2)[CuL_2] + \\ \Delta H'[CuHL] + (\Delta H_1' + \Delta H_2')[Cu(HL)_2] + \\ (\Delta H_1' + \Delta H_2'')[Cu(HL)L]\end{array}}{\begin{array}{l}[CuL] + 2[CuL_2] + [CuHL] + \\ 2[Cu(HL)_2] + 2[Cu(HL)L]\end{array}} \quad (21)$$

formation and protonation constants, canceling like terms, and rearranging, the form of the function shown

in eq 22 is obtained. The problem is, given $[([L]_i,$

$$\overline{\Delta H} = \frac{\Delta H_1 K_1 + (\Delta H_1 + \Delta H_2) K_1 K_2 [L] + \Delta H_1' K_1' [H^+]/K_{a_3} + (\Delta H_1' + \Delta H_2') \times K_1' K_2' [H^+]^2 [L]/(K_{a_3})^2 + (\Delta H_1' + \Delta H_2'') K_1' K_2'' [H^+][L]/K_{a_3}}{K_1 + 2K_1 K_2 [L] + K_1' [H^+]/K_{a_3} + 2K_1' K_2' [H^+]^2 [L]/(K_{a_3})^2 + 2K_1' K_2'' [H^+][L]/K_{a_3}} \quad (22)$$

$[H^+]_i, \overline{\Delta H}_i)$: $i = 1, 2, \ldots, N]$, where N is the number of data points, determine the set of enthalpy values (ΔH_1, ΔH_2, $\Delta H_1'$, $\Delta H_2'$, $\Delta H_2''$) which minimize

$$F(\Delta H_1, \Delta H_2, \Delta H_1', \Delta H_2', \Delta H_2'') = \sum_{i=1}^{N} [\overline{\Delta H}_i - f([L]_i, [H^+]_i; \Delta H_1, \Delta H_2, \Delta H_1', \Delta H_2', \Delta H_2'')]^2 \quad (23)$$

where $f([L]_i, [H^+]_i; \Delta H_1, \Delta H_2, \Delta H_1', \Delta H_2', \Delta H_2'')$ is the right-hand side of eq 22.

The hydrogen ion concentration, $[H^+]$, was estimated from pH readings; the free ligand concentration, [L], was calculated by the iterative procedure described for the stability constants. K_1, K_2, K_1', K_2', K_2'', K_{a_1}, K_{a_2}, and K_{a_3} were defined above.

The calculation procedure was as follows. Each enthalpy value was varied over a given interval and F (eq 23) was calculated at specific increments. The enthalpy value which gave the smallest deviation was stored and used in subsequent calculations of F. The iterations were continued until the desired fit of eq 22 was attained. In practice the iterations were continued until the largest deviation from the $\overline{\Delta H}$ curve was less than 0.1 kcal/mol over the complete enthalpy titration range. The initial heat values used were approximated from the preceding studies of histamine, histidine methyl ester, imidazolepropionic acid, and the homologous series of amino acids reported earlier.[1]

The final enthalpy values listed in Table VI represent

Table VI. Heats of Reaction for Histidine

$-\Delta H_1$[a]	$-\Delta H_2$[b]	$-\Delta H_1'$	$-\Delta H_2''$
11.57 (±0.05)[c]	9.75 (±0.23)	11.19 (±0.07)	14.98 (±0.24)

[a] In kcal/mol. [b] Stack and Skinner[19] report −21.0 kcal/mol for $\Delta H_1 + \Delta H_2$ (calorimetrically); Raju and Mathur[18] report −22.1 kcal/mol for $\Delta H_1 + \Delta H_2$ (temperature variation). [c] Errors represent standard deviation for 11 independent enthalpy titrations.

11 independent enthalpy titrations performed under varying conditions of concentration and pH. Comparison is made with available literature values. A value of $\Delta H_2'$ was not calculated owing to the low concentration of the species $Cu(HL)_2^{2+}$.

Discussion

Thermodynamics of Deprotonation. The thermodynamic parameters for the deprotonation of the ligands histidine, histamine, histidine methyl ester, and imidazolepropionic acid are summarized in Table VII. Phenylalanine,[1] similar structurally to histidine, is included for comparison with the "simple" amino acids.

Table VII. Thermodynamics of Deprotonation

Ligand	Functional group	pK	ΔH, kcal/mol	ΔS, eu
Phenylalanine	Amino	9.02	10.32	6.6
Histidine	Imidazole	5.39	7.57	−0.7
methyl ester	Amino	7.37	9.97	0.3
Histamine	Imidazole	6.25	7.42	3.7
	Amino	9.97	12.55	3.5
Imidazole-	Carboxyl	3.96	−0.01	18.2
propionic acid	Imidazole	7.57	8.82	5.1
Histidine	Carboxyl	2.02	0.72	6.8
	Imidazole	6.16	7.01	4.7
	Amino	9.21	10.43	7.1

Reasonable agreement with the literature for these systems is evident from Tables I and IV.

The heat of deprotonation for the amino group of histidine is similar in magnitude to the simple amino acids (*i.e.*, phenylalanine). Histidine methyl ester, however, appears to have a very low pK_a for the amino group. This appears to be due primarily to an entropy effect. This is not too surprising since the charges on the two-protonated ligands are different (1+ for the ester *vs.* 0 (zwitterion) for histidine). Histamine is significantly more basic than the amino acids. This appears to be primarily an enthalpy effect.

The values obtained for imidazole deprotonation are similar; however, the pK_a recorded for histidine methyl ester is again anomalously low. This again appears to be an entropy effect. The heats of deprotonation for the carboxyl groups of histidine and imidazolepropionic acid are comparable, but a large difference in pK_a's, as reflected by the entropy terms, is evident. The values obtained for imidazolepropionic acid approximate those obtained for its constituents, propionic acid[24] (pK_a = 4.88, ΔH = 0.08, ΔS = −22.6) and imidazole[14,25] (pK_a = 7.09, ΔH = 8,9, ΔS = −3), indicating that the increased chain length allows the two functional groups to act independently.

Thermodynamics of Complexation. Histidine Derivatives. The results of the thermodynamic studies of the histidine related compounds are summarized in Table VIII. Phenylalanine[1] is again included to provide an example of amino–carboxyl chelate bonding. Agreement with the literature for the stability constants is found for histidine and its derivatives (Tables II and III). Conditions consistent with those of the present study were generally used. Larger discrepancies appear in the comparison of the heats of complexation (Table V). The enthalpy values determined in the pres-

Table VIII. Thermodynamics of Complexation

Ligand	Log K_1	$-\Delta H_1$	ΔS_1	Log K_2	$-\Delta H_2$	ΔS_2
Phenylalanine	7.51	5.24	16.8	6.74	5.77	11.5
Imidazole-propionic acid	4.46	3.65	8.2	4.03	5.15	1.2
Histidine methyl ester	8.46	11.04	1.7	5.92	8.16	−0.3
Histamine	9.56	13.48	−1.4	6.37	10.84	−7.2
Histidine	10.01	11.57	7.0	8.01	9.75	4.0

(24) W. J. Canady, H. M. Papee, and K. J. Laidler, *Trans. Faraday Soc.*, **53**, 502 (1957).
(25) I. Wadso, *Acta Chem. Scand.*, **16**, 479 (1962).

ent investigation were the only ones determined calorimetrically and are believed to be more accurate.

Histidine methyl ester and histamine form chelates containing an amino nitrogen and an imidazole nitrogen. The heats of reaction are considerably more exothermic than those of other members of the series. The ΔH values for histidine indicate that an N–N chelate bond is being formed. The enthalpy values listed for histamine are larger than those given for histidine or its ester, which can be expected from the base strengths. The reaction of imidazolepropionic acid with copper(II) is the least exothermic of the series and reflects the less basic character of the imidazole group compared to amino coordination (phenylalanine). Assuming that the heats of reaction can be related to bond strength, the following order of chelate stability can be derived from Table VIII, where I, A, and C refer to imidazole, amino, and carboxyl groups, respectively: I–A $\gg$ A–C > I–C.

The free energy values, which reflect the overall stability of the complex, follow generally the order established above. The entropy change accompanying the complexation reaction now becomes important since log K_2 for phenylalanine is actually larger than for either of the neutral N–N bidentate ligands (histidine methyl ester and histamine). The large positive entropy values for the anionic ligand (phenylalanine) have been ascribed earlier[1] to large differences in hydrational energies accompanying the neutralization of charge at the metal. This effect does not occur with the neutral ligands and the negative entropy terms are the result of competing cratic and conformational terms. This apparent large difference in entropy between anionic and neutral ligands results in comparable formation constants, indicating that free energy values alone can lead to erroneous conclusions regarding metal–ligand bond strengths unless closely related systems are compared.

Imidazolepropionic acid forms the least stable copper complex of the systems studied. Lower basicity and the necessity of forming a seven-membered chelate ring contribute to the reduced stability.

Histidine. The anomalous behavior of the copper(II)—histidine system has been explained earlier by assuming that a protonated form of the ligand was contributing to the formation of the complex. Leberman and Rabin,[6] Perrin,[7] and Perrin and Sharma[12] assumed that the amino group was protonated, since it is the most basic, and that the ligand was chelating through the imidazole and carboxyl groups. They were able to explain their data adequately by assuming that this form of the ligand and the unprotonated form were the only bonding species. The calculated formation constants for the monoprotonated species (Table III) are consistent with the known coordination ability of imidazole[26] (log K_1 = 4.00), which indicated that this was the bonding species.

A potentiometric study by Sarkar and Wigfield[27] has indicated that the bonding sites of the histidine molecule are the amino and carboxyl groups only. This conclusion was based on the number of protons displaced during the formation of the bis(histidinato)-copper(II) complex; however, the existence of the mixed protonated complex $Cu(HL)L^+$ was not mentioned. The complexation reaction was studied over a relatively high pH range (5.5–10.0) to ensure complete coordination. The bonding sites for the mono complexes were not considered.

X-Ray studies also have presented conflicting views. The structure of bis-L-histidinecopper(II) dinitrate dihydrate[28] (obtained at pH 3.7) has shown that each histidine molecule is coordinated through the amino nitrogen and a carboxylate oxygen with the imidazole group turned away from the copper. However, the histidine molecule in the mixed complex L-histidinato-L-threoniatoaquocopper(II) hydrate[29] (obtained at physiological pH) was shown to bond in the plane with its amino and imidazole groups. A carbonyl oxygen resided in an irregular axial position. The differences in the bonding of the solid complexes are apparently a function of the pH of the crystallizing solution. Lattice effects, however, prevent a direct correlation between the bonding sites of the ligand in the solid complex and in aqueous solution.

Spectroscopic studies in aqueous solution have exhibited similar complications. Ultraviolet and infrared (D_2O) spectra of the bis complex were interpreted by Sarkar and Wigfield[27] to indicate amino and carboxyl chelation. Carlson and Brown,[30] from infrared studies in D_2O, concluded that bidentate copper(II)–histidine chelates were being formed in the region pD 3.3–5.1 with the imidazole and carboxyl groups of the ligand. No amino coordination was observed in this region. A monodentate complex was said to be formed below this region with the carboxyl group coordinating. A tridentate complex was thought to be formed at high pD values. Wellman, *et al.*,[31] on the basis of ORD and CD measurements, have proposed that histidine was bonding as a tridentate ligand only.

The results of the present study, however, indicate that the histidine molecule is bonding with its amino and imidazole nitrogens in the pH range of this investigation (2.8–10.0). The thermodynamic values (particularly ΔH) obtained for the bonding of anionic histidine to copper(II) (Table VIII) are similar to those for histamine and histidine methyl ester. A larger entropy term for histidine can be argued in terms of charge neutralization of the complex and subsequent hydrational disorders. The enthalpy values are much too exothermic to be the result of either amino–carboxyl (phenylalanine) or imidazole–carboxyl (imidazolepropionic acid) chelation. Direct participation of the carboxyl group in the histidine complexes cannot be determined because of its small contribution to the enthalpy term. A ΔS value smaller than that for phenylalanine may indicate, however, that the carboxyl group is not coordinated to the copper and is free to interact with the solvent.[32]

The thermodynamic results for the monoprotonated histidine systems are presented in Table IX. Results are also included for the reaction

(26) J. T. Edsall, G. Felsenfeld, D. S. Goodman, and F. R. N. Gurd, *J. Amer. Chem. Soc.*, **79**, 5859 (1957).
(27) B. Sarkar and Y. Wigfield, *J. Biol. Chem.*, **242**, 5572 (1967).
(28) B. Evertsson, *Acta Crystallogr., Sect. B*, **25**, 30 (1969).
(29) H. C. Freeman, J. M. Gass, M. J. Healy, R. P. Martin, and C. E. Nockolds, *Chem. Commun.*, 225 (1969).
(30) R. H. Carlson and T. L. Brown, *Inorg. Chem.*, **5**, 268 (1966).
(31) K. M. Wellman, T. G. Mecca, W. Mungall, and C. R. Hare, *J. Amer. Chem. Soc.*, **89**, 3647 (1967).
(32) G. H. Nancollas and D. J. Poulton, *Inorg. Chem.*, **8**, 680 (1969).

Table IX. Thermodynamics of Complexation of Copper(II) with Monoprotonated Histidine

Reaction	Amino group protonated			Carboxyl group protonated		
	Log K	$-\Delta H$	ΔS	Log K	$-\Delta H$	ΔS
$Cu^{2+} + HL \rightleftarrows CuHL^{2+}$	4.37	11.19	−17.6	9.37	11.19	5.3
$CuL^{+} + HL \rightleftarrows Cu(HL)L^{+}$	4.60	15.32	−30.4	9.60	15.32	−7.5
$CuHL^{2+} + L^{-} \rightleftarrows Cu(HL)L^{+}$				10.13	14.98	−3.9

$$CuL^{+} + HL \rightleftharpoons Cu(HL)L^{+} \qquad K_2''' = \frac{[Cu(HL)L^{+}]}{[CuL^{+}][HL]} \quad (24)$$

The calculation procedure was similar to that already described with the exception that eq 24 was substituted for eq 8 in all instances. The ΔH values again indicate amino–imidazole chelation. The carboxyl group must then be protonated. If the amino group is considered protonated, unrealistic entropy values are obtained which indicate that improper values for the formation constant had been chosen. Since the equilibrium constant expression (6) can be rewritten

$$K_1' = \frac{[CuHL^{2+}]K_{a_3}}{[Cu^{2+}][L^{-}][H^{+}]} \quad (25)$$

it is obvious that the value of K_1' obtained will be directly proportional to the value of K_{a_3} used. K_{a_3} is the protonation constant for the removal of the third proton from histidine. A similar expression can be written for K_2'''. The discussion above suggests that the carboxyl group is protonated; therefore, the "microconstant" for the removal of a proton from the carboxyl group of the neutral charged ligand must be used. Since the concentration of this species is always negligibly small, its value cannot be determined by an ordinary potentiometric titration. The value of this constant cannot be the same as the value determined for K_{a_1} (Table I), since whether or not the adjacent amino group is protonated will have a pronounced effect on the acidity of the carboxyl group. A value for this constant can be estimated, however, by analogy to a study of tyrosine (structurally similar to histidine), where all the individual protonation "microconstants" were determined by a combination of potentiometric and spectrophotometric measurements. Martin, *et al.*,[33] found that if the α-amino group was not protonated, the pK_a for carboxyl deprotonation increased by 2.19 log units at an ionic strength of 0.16. Assuming that a similar effect would occur with histidine a pK value of 4.21 is obtained for the deprotonation of the carboxyl group of the neutral histidine molecule.[34] This would represent an increase of 5.00 log units for the acid dissociation constant used in the evaluation of K_1' and K_2''' (*i.e.*, eq 24) since the pK_a for amino dissociation was found to be 9.21. The new values calculated for K_1' and K_2''', along with the corresponding entropy values, are included in Table IX. Entropy terms consistent with the results of Table VIII are now obtained. Also presented are the thermodynamic parameters for the formation of the mixed complexes *via* eq 8.

The log K and ΔH values for the formation of the mixed complex $Cu(HL)L^{+}$ appear to be large compared to the results presented in Table VIII for log K_2 and ΔH_2 for the N–N chelates, but rather approximate the values obtained for the formation of the mono(histaminato)copper(II) species. This indicates that an unusually stable complex is being formed.

The acid dissociation constant for the deprotonation of the 1:1 complex which is described by the equilibrium

$$CuHL^{2+} \rightleftharpoons CuL^{+} + H^{+} \quad (26)$$

can be calculated by appropriate combination of the acid dissociation and formation constants.

$$K_{a_3}' = \frac{K_1 K_{a_3}}{K_1'} \quad (27)$$

The computed pK_{a_3} (3.57) is lower than the estimated value (4.21) for deprotonation of the neutral histidine molecule. This is predicted, since metal coordination is expected to withdraw electron density from the carboxyl group, making it less basic. It is, however, higher than the measured value (2.02) for the completely protonated form of the ligand. Leberman and Rabin[6] obtained a value of 3.81 for this reaction. The very low pK_a for amino dissociation was explained by electronic interactions accompanying the complex formation.

The enthalpy and entropy values for this reaction can also be estimated by subtracting eq 6 from the sum of eq 3 and 4. Assuming eq 3 is amino deprotonation and assigning a value of +10.43 kcal for ΔH (histidine, Table VII), a ΔH value of +10.05 kcal and a ΔS value of +17.3 eu are obtained, which is unrealistic for amino deprotonation. However, if carboxyl deprotonation is assumed for eq 3 and a ΔH value of −0.01 kcal (imidazolepropionic acid, Table VII) is assigned, a ΔH value of −0.39 kcal and a ΔS value of −17.6 eu are calculated which are very similar to the carboxyl deprotonation of imidazolepropionic acid.

In conclusion, the results of the enthalpy titrations strongly suggest that both the unprotonated and the monoprotonated form of the histidine molecule bond to copper(II) through its amino and imidazole groups. The carboxyl group must then be protonated in the monoprotonated complexes. The preceding discussion emphasizes the value of complementary enthalpy data for determining bond sites.

(33) R. B. Martin, J. T. Edsall, D. B. Wetlaufer, and B. R. Hollingworth, *J. Biol. Chem.*, **233**, 1429 (1958).

(34) It is possible to estimate the ionization constant for a carboxylic acid ionization from a neutral histidine molecule without recourse to tyrosine. As shown in ref 33, from the properties of a cyclic system, the product of all histidine ionization constants divided by the product of histidine methyl ester constants yields the desired constant. The pK value 4.64 obtained in this manner does not alter any of the qualitative conclusions made above.