Thermodynamics of Amino Acid–Copper(II) Complexes

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Log *K* and calorimetrically determined enthalpy values are reported for a series of amino acids upon combination with H^+ and Cu^{+2}. The amino acids studied are alanine, α-amino-*n*-butyric acid, valine, leucine, phenylalanine, and tryptophan, which are all related with respect to substitution at the β-carbon atom. An additional bonding site is proposed for the amino acid tryptophan (other than the carboxyl and α amino group) in the formation of the bistryptophan-copper(II) complex. All values were determined at 25° C. and at a constant ionic strength of 0.16.

THE BASE STRENGTHS and metal-ion bonding of the amino acids reported here have been studied by a number of workers (*2, 3, 9, 17, 18*), but direct calorimetry has been used by relatively few researchers to examine the solution thermodynamics of amino acid complexes. Sturtevant (*21*) studied the protonation of alanine calorimetrically. More recently Anderson *et al.* (*2*) reported on protonation and complexation reactions of alanine and phenylalanine. Stack and Skinner (*20*) report over-all heats of complexation for a series of amino acids and related compounds, including alanine. This paper presents the potentiometrically determined acid dissociation constants and copper(II) formation constants as well as the corresponding enthalpies determined by direct calorimetry. The results of this study will be used as an aid in determining bonding sites for more complex systems.

EXPERIMENTAL

Materials. Carbonate-free sodium hydroxide was obtained from the Bio-Rad Laboratories and standardized by titration with potassium acid phthalate. Nitric acid solutions were prepared from Baker analyzed reagent grade acid and standardized by titration with sodium hydroxide. All inorganic salts were of reagent grade. The 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.

The amino acids L-alanine, DL-α-amino-*n*-butyric acid, L-valine, L-leucine, L-phenylalanine, and L-tryptophan were obtained as the free base (zwitterion) from the Nutritional Biochemicals Corp. and used without further purification after drying at 110° C. for 1 hour. Their purity was estimated to be greater than 99.5% from potentiometric end points.

Potentiometric Titrations. A Beckman Zeromatic pH meter equipped with standard glass and calomel electrodes was used for the determination of the stability constants and protonation constants. The titrations were performed in a constant temperature cell kept at 25.0° ± 0.1° C. by circulating water from a constant temperature bath. Carbon dioxide absorption was prevented by passing nitrogen, presaturated by bubbling through $0.16M$ KNO_3, over the surface of the titration solution.

The titrations were performed by adding standard sodium hydroxide ($\sim 5 \times 10^{-2}M$) first to a solution containing amino acid ($\sim 8 \times 10^{-2}M$) and then to an identical solution containing a 2 to 3:1 mole ratio of amino acid to copper. The titrations were performed consecutively, so that the acid constants and the stability constants would be determined under nearly identical conditions. Solutions of the amino acids were prepared immediately before use to minimize the possibility of dipeptide formation.

Calorimetric Titrations. DESIGN OF CALORIMETER. Since the formation of the bis amino acid–copper(II) complexes proceeds in a stepwise manner, a calorimeter was designed so that aliquots of titrant could be added successively and heats of reaction could be measured during the complete reaction sequence. For this requirement the calorimeter illustrated in Figure 1 was designed and tested.

The 150-ml. reaction vessel, *D*, was constructed from thin-walled (1-mm.) glass and supported by means of a screw lid assembly, *A*, which allowed easy access to the calorimeter contents. A constant speed 200-r.p.m. stirring motor was placed to one side of the calorimeter and connected by a belt to the stirrer, *E*. A measured amount of acetone in a closed-end tube, *I*, was evaporated by air to provide for cooling.

The buret assembly consisted of an outer glass tube containing a reservoir, *B*, connected at the bottom by capillary tubing which extends above the top of the calorimeter solution. The titrant to be added was introduced by a microburet, *C*, the tip of which extended down into the reservoir. After a known amount of titrant was added to the reservoir and allowed to reach thermal equilibrium, it was gradually forced into the reaction solution by a slight positive pressure exerted by an all-glass syringe connected to the assembly via a side arm, *F*. The dispensing operations were calibrated and found to deliver a volume of solution reproducible to ±0.012 ml. A correction of 0.017 ml. was made for the first addition of titrant to account for the solution which adheres to the buret walls. Volumes of solution normally added range from 1.5 to 2.5 ml.

The temperature-measuring device, *H*, was a glass-coated thermistor (Veco, 25A6, 500-ohm). It was immersed in mineral oil, used as a heat transfer medium, in thin-walled glass tubing. The temperature-measuring circuit was similar to that described by Wang (*23*). The thermistor formed one arm of a Wheatstone bridge. The other three arms consisted of three 500-ohm precision resistors. A 6-volt storage battery supplied the bridge with a current of approximately 0.6 ma. after passing through a 10,000-ohm

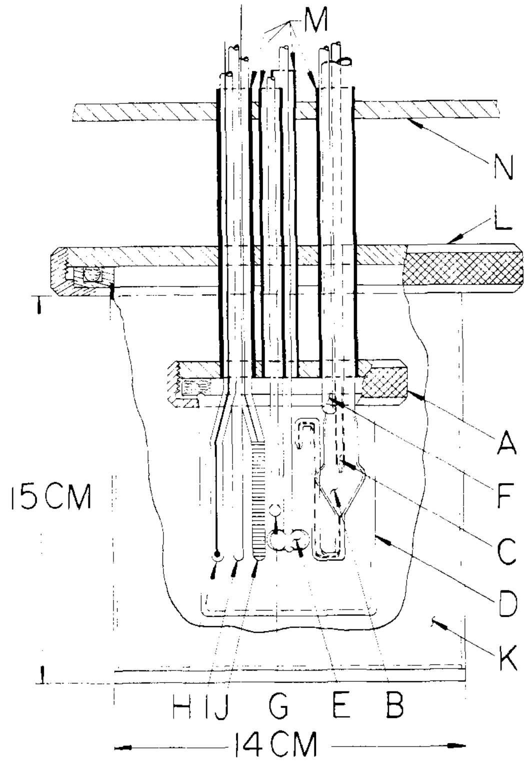

Figure 1 Schematic of the calorimeter

A. Calorimeter vessel support (screw lid)
B. Titration device
C. Buret tip
D. Calorimeter vessel (150-ml. glass)
E. Stirrer
F. Side arm (leads to glass syringe)
G. pH electrode
H. Thermistor
I. Cooling tube
J. Heater
K. Brass container
L. Screw lid (contains O-ring seal)
M. Brass tubes (entering calorimeter)
N. Bakelite support

slide wire resistor. The output at the bridge was connected to a Leeds & Northrup 7553 Type K-3 potentiometer, which was used to balance the bridge potential at the operating temperature. The unbalanced potential resulting from temperature change in the calorimeter was amplified by a d.c. microvolt indicating amplifier (Leeds & Northrup) and was recorded by a potentiometric chart recorder (Minneapolis-Honeywell), equipped with a printing mechanism which indicated the potential every 15 seconds. The potentiometer and indicating amplifier system were also used in measuring the current through the Wheatstone bridge and the electrical heater.

The heater, *J*, used for electrical calibration, was made of coiled No. 38 gage insulated manganin wire, wound on an insulator to a resistance of 73.95 ohms. Insulated copper leads were connected to the resistance wire by thermal free solder. The heater was immersed in paraffin oil in the thin-walled glass tubing. A dummy heater was made similarly and used to stabilize the heater current. The heater current was supplied by a 6-volt storage battery which was adjusted to supply approximately 27 ma. by a 150-ohm slide wire resistor. The time of heating was measured to 0.1 second by an electric timer which was activated simultaneously with the heater by means of a 4 PDT switch.

The pH of the calorimeter solution could also be monitored by using a pH electrode, *G*. The electrode (Sargent S-30070-10 combination miniature) was connected to a pH meter outside the bath.

The titration vessel was enclosed in a nickel brass container, *K*. This provided a "constant temperature environment" when immersed in the water bath. This design (semiadiabatic) allows for reasonably rapid thermal equilibrium after each addition of titrant. The container was sealed at the top by a screw lid assembly, *L*, containing an O-ring seal. The calorimeter vessel and brass container were supported from above by three concentric brass tubes, *M*, which also housed the working parts of the calorimeter. The whole calorimeter assembly was supported by a Bakelite platform, *N*, above a water bath maintaining a temperature to within ±0.001° C. The bridge output, which corresponded to 25.000° C., was verified by comparison with a certified NBS thermometer. The bridge response was also shown to be linearly dependent on temperature for the complete range of operation.

OPERATION OF CALORIMETER. The calorimeter was immersed in the constant temperature bath at least 12 hours before each enthalpy titration. The temperature-measuring circuit was turned on at least 1 hour before each heat run to stabilize the electronics. The 4 PDT switch in the heater circuit was connected to the dummy heater also at least 1 hour before each experiment to stabilize the heater current.

The calorimeter was calibrated by measuring the heat of neutralization of sodium hydroxide with nitric acid, using the value of 13.40 kcal. per mole. This value is reported at an ionic strength of 0.016 by Hale *et al.* (*8*).

The heats of protonation were determined by adding a solution of nitric acid (3 to 8 × $10^{-1}M$) to a solution of the nearly completely deprotonated amino acid (1 to 8 × $10^{-2}M$) or alternatively by adding sodium hydroxide (3 to 8 × $10^{-1}M$) to a solution of the nearly completely protonated amino acid (1 to 8 × $10^{-2}M$). Both procedures gave equivalent results.

The heats of complexation were measured by adding the anionic form of the ligand (5 to 10 × $10^{-2}M$) to a solution of copper nitrate (4 to 8 × $10^{-3}M$).

Two or more calorimetric titrations were performed for all of the above systems. The accuracy of the measured heats, expressed as one standard deviation of the mean, is approximately 0.5%.

RESULTS

Potentiometric Titrations. ACID DISSOCIATION CONSTANTS. The equilibrium involved for the dissociation of the zwitterion can be represented by Equation 1. The values for the deprotonation

$$HL^{\pm} \rightleftharpoons L^{-} + H^{+} \quad K_a = \frac{[L^{-}][H^{+}]}{[HL^{\pm}]} \tag{1}$$

constants were calculated by conventional means, utilizing the measured pH and initial concentrations. The acid dissociation constants for deprotonation of the amino group only are presented in Table I, since it was estimated that less than 1% of the ligand is doubly protonated under the conditions of the stability constant titrations. Comparison is made with appropriate literature values.

STABILITY CONSTANTS. The stepwise formation of the bis complexes can be expressed by the following equilibria:

$$\mathrm{Cu}^{+2} + L^{-} \rightleftharpoons \mathrm{Cu}L^{+} \quad K_1(\beta_1) = \frac{[\mathrm{Cu}L^{+}]}{[\mathrm{Cu}^{2+}][L^{-}]} \tag{2}$$

$$\mathrm{Cu}L^{-} + L^{-} \leftrightarrows \mathrm{Cu}L_2 \quad K_2 = \frac{[\mathrm{CuL}_2]}{[\mathrm{Cu}L^{+}][L^{-}]} \tag{3}$$

The over-all reaction is given by

$$\mathrm{Cu}^{2+} + 2L^{-} \leftrightarrows \mathrm{Cu}L_2 \quad \beta_2 = \frac{[\mathrm{Cu}L_2]}{[\mathrm{Cu}^{2+}][L^{-}]^2} \tag{4}$$

It was assumed that these were the principal equilibria involved. Conditions were adjusted to minimize known competing reactions.

The $\bar{n}$ method was chosen for the calculation of the stability constants (*15*), where $\bar{n}$ is defined as the average number of ligands bound per metal ion.

$$\bar{n} = \frac{[\mathrm{Cu}L^+] + 2[\mathrm{Cu}L_2]}{C_m} \tag{5}$$

where

$$C_m = [\mathrm{Cu}^{2+}] + [\mathrm{Cu}L^+] + [\mathrm{Cu}L_2] \tag{6}$$

Substituting Equations 2 and 4 and canceling the free metal ion concentration, Equation 7 results.

$$\bar{n} = \frac{\beta_1[L^-] + 2\beta_2[L^-]^2}{1 + \beta_1[L^-] + \beta_2[L^-]^2} \tag{7}$$

This equation can be arranged in the form of a linear equation.

$$\frac{(1-\bar{n})}{\bar{n}}[L^-] = -\frac{\beta_2}{\beta_1}\frac{(2-\bar{n})}{\bar{n}}[L^-]^2 + \frac{1}{\beta_1} \tag{8}$$

A plot of

$$\frac{1-\bar{n}}{\bar{n}}[L^-] \text{ } vs. \text{ } \frac{2-\bar{n}}{\bar{n}}[L^-]^2$$

yields a straight line of slope $-\beta_2/\beta_1$ and intercept $1/\beta_1$. The formation constants, β_1 and β_2, were calculated from the slope and intercept by fitting the experimental $\bar{n}$ and $[L^-]$ data to Equation 8. A linear least squares treatment was used with the aid of a 1710 IBM computer.

The $\bar{n}$ and $[L^-]$ values were calculated from the experimental data by means of the following equations.

$$\bar{n} = \frac{C_{HL} - [L^-] - [HL^{\pm}]}{C_m} \tag{9}$$

$$[L^-] = \frac{K_a[HL^{\pm}]}{[H^+]} = \frac{K_a(C_{HL} - C_B + [OH^-] - [H^+])}{[H^+]} \tag{10}$$

C_{HL}, C_B, and C_m are the total concentration of zwitterion, base, and copper(II) ion, respectively.

The values for the stability constants obtained in this investigation are given in Table II along with available

Table I. Thermodynamics of Deprotonation

$$HL^{\pm} \rightleftarrows H^+ + L^-$$

	pK		ΔH		−ΔS	
Phenylalanine	9.02[a]	9.31[b]	10.32[a](C)[c]	10.67[b](C)	6.6[a]	
	9.13[d]	9.13[e]	10.30[f] (T)[g]			
	9.15[f]					
Tryptophan	9.28[a]	9.55[h]	10.65[a] (C)		6.7[a]	
Valine	9.44[a]	9.62[d]	10.97[a] (C)	10.70[i] (T)	6.4[a]	8.4[i]
	9.72[i]	9.61[j]				
Leucine	9.54[a]	9.75[i]	10.73[a] (C)	10.90[i] (T)	7.6[a]	8.0[i]
	9.60[d]					
α-Amino-*n*-butyric acid	9.56[a]	9.33[k]	10.73[a] (C)	10.80[i] (T)	7.7[a]	8.8[i]
	9.83[i]					
Alanine	9.60[a]	9.87[d]	10.62[a] (C)	10.60[f] (T)	8.3[a]	8.0[i]
	9.87[l]	9.41[k]	11.00[i] (T)	10.80[l] (T)	7.4[m]	9.8[f]
	9.78[f]	9.89[m]	10.80[d] (C)	11.3[m] (C)		
	9.97[h]	9.86[i]				

[a] Present study. [b] (*2*). [c] Calorimetrically determined. [d] (*12*). [e] (*10*). [f] (*9*). [g] Temperature variation method. [h] (*1*). [i] (*19*). [j] (*11*). [k] (*17*). [l] (*14*). [m] (*3*).

Table II. Thermodynamics of Complexation

	$\mathrm{Cu}^{2+} + L^- \rightarrow \mathrm{Cu}L^+$			$\mathrm{Cu}L^+ + L^- \rightarrow \mathrm{Cu}L_2$			$\mathrm{Cu}^{2+} + 2L^- \rightarrow \mathrm{Cu}L_2$		
	Log K_1	$-\Delta H_1$	ΔS_1	Log K_2	$-\Delta H_2$	ΔS_2	Log β_2	$-\Delta H_T$	ΔS_T
Phenylalanine	7.51[a]	5.24[a](C)[b]	16.8[a]	6.74[a]	5.77[a](C)	11.5[a]	14.25[a]	11.01[a](C)	28.3[a]
	8.3[c]	5.10[c](T)[d]	20.7[c]	6.9[c]	6.40[c](T)	10.7[c]	15.2[c]	11.50[c](T)	31.4[c]
	8.25[e]	5.30[e](C)	19.8[e]	7.13[e]	6.4[e](C)	13.7[e]	15.38[e]	11.70[e](C)	33.5[e]
	7.74[f]			6.90[f]			14.9[g]		
							14.66[h]		
							14.64[f]		
Tryptophan	7.80[a]	5.48[a](C)	17.3[a]	7.30[a]	7.21[a](C)	9.2[a]	15.10[a]	12.69[a](C)	26.5[a]
							15.9[g]		
Valine	7.80[a]	5.46[a](C)	17.4[a]	6.70[a]	5.84[a](C)	11.1[a]	14.50[a]	11.30[a](C)	28.5[a]
	7.92[i]			6.5[i]			14.44[i]	20.50[j](T)	0[j]
							14.76[j]		
							15.10[g]		
Leucine	7.90[a]	5.55[a](C)	17.6[a]	6.70[a]	5.90[a](C)	10.9[a]	14.60[a]	11.45[a](C)	28.4[a]
	7.89[i]			6.45[i]			14.34[i]		
	8.35[k]			7.00[k]			15.35[k]		
α-Amino-*n*-butyric acid	7.84[a]	5.52[a](C)	17.3[a]	6.64[a]	5.98[a](C)	10.3[a]	14.48[a]	11.50[a](C)	27.7[a]
	8.21[l]	5.40[l](T)	19.2[l]	6.72[l]	5.30[l](T)	13.0[l]	14.93[l]	10.70[l](T)	32.2[l]
	8.0[m]			6.5[m]			14.5[m]		
Alanine	7.84[a]	5.62[a](C)	17.0[a]	6.64[a]	6.00[a](C)	10.3[a]	14.48[a]	11.62[a](C)	27.3[a]
	8.18[i]	5.60[c](T)	20.3[c]	6.65[i]	6.20[c](T)	10.9[c]	14.83[i]	11.80[c](T)	31.2[c]
	8.29[l]	4.90[l](T)	21.2[l]	6.72[l]	5.80[l](T)	11.3[l]	15.01[l]	10.70[l](T)	32.5[l]
	8.58[c]	4.51[n](C)	23.9[n]	6.98[n]	5.24[n](C)	14.3[n]	15.52[h]	9.75[n](C)	38.2[n]
	8.54[n]			7.5[m]			15.1[g]	11.90[o](C)	
	8.1[m]			6.86[c]			14.6[m]		
	8.5[p]			6.86[p]			15.44[c]		
							15.37[p]		

[a] Present study. [b] Calorimetrically determined. [c] (*9*). [d] Temperature variation method. [e] (*2*). [f] (*5*). [g] (*1*). [h] (*10*). [i] (*12*). [j] (*11*). [k] (*7*). [l] (*16*). [m] (*17*). [n] (*3*). [o] (*20*). [p] (*13*).

literature values. The accuracy of the values is estimated to be ± 0.1 log unit.

Calorimetric Titrations. Heats of Deprotonation. Heats observed for the deprotonation of the amino group were corrected for heat due to competing neutralization reactions. These were calculated from the stoichiometries and the reported heat of neutralization of a strong acid with a strong base (*8*). These results are summarized in Table I along with available literature values.

Heats of Complexation. All of the species depicted in Equations 2 and 3 must be considered for most of the titration range, since the successive formation constants K_1 and K_2 are generally close. This makes the assignment of individual heats difficult; therefore the following linear equation was used to treat the data. $\overline{\Delta H}$ is defined as the average heat per mole of ligand complexed. ΔH_1 and ΔH_2 refer to Equilibria 2 and 3, respectively.

$$\overline{\Delta H} = \frac{\Delta H_1[CuL^+] + (\Delta H_1 + \Delta H_2)\,[CuL_2]}{[CuL^+] + 2[CuL_2]} \qquad (11)$$

Substituting Equations 2 and 3 into 11, Equation 12 results.

$$\overline{\Delta H} = \frac{\Delta H_1 + (\Delta H_1 + \Delta H_2)\,K_2[L^-]}{1 + K_2[L^-]} \qquad (12)$$

Equation 12 can be arranged in linear form

$$\overline{\Delta H}\,(1 + \theta) = \Delta H_2\theta + \Delta H_1 \qquad (13)$$

where

$$\theta = \frac{K_2[L^-]}{1 + K_2[L^-]}$$

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

Corrections were applied to the observed heat for competing protonation reactions (generally less than 0.1%). The free ligand concentration, $[L^-]$, was calculated from the stoichiometries and the previously determined protonation and stability constants. The data are collected in Table II, where available literature values are compared.

DISCUSSION

Protonation Reactions. Log K, ΔH, and ΔS values for the deprotonation of the amino nitrogen are shown in Table I along with other reported results. The amino acids are listed in order of increasing base strength. The agreement is reasonable if the different media and modes of determination are considered. Activity coefficients for the generally higher ionic strength (0.16) used in this study can be calculated by the empirical equation used by Datta and Grzybowski (*6*) for the proton-glycine system to compare the data presented here to the deprotonation constants obtained at low ionic strength by other workers. A value of 0.145 is calculated for log γ by this equation. If 2 log γ is added to the observed pK_a (the activity coefficient of the zwitterion is assumed to be 1) to correct to $\mu = 0$, consistent values for the deprotonation constants are obtained.

Complexation Reactions. Table II lists the log K, ΔH, and ΔS values for the reactions with copper(II) ion. The ligands are listed in the same order as above. Again, reasonable agreement with the literature is evident if differences in ionic strength are considered. Stability constant values can be corrected to zero ionic strength by an empirical equation used by Brannan *et al.* (*4*) for metal-glycine systems. If the calculated correction factor (0.26 log unit) is added to each of the log K values in Table II, stability constants consistent with literature values obtained at low ionic strength are found. Reasonable agreement is also obtained for the available enthalpy values.

The results in Table II show that (except for tryptophan discussed below) all of the amino acids studied have essentially the same ability to form complexes with copper(II). The fact that ΔH_2 is more exothermic than ΔH_1 for the whole series, while ΔS_1 is much more positive than ΔS_2, has been ascribed earlier (*3*) to the large difference in hydrational energies of the Cu_{aq}^{+2} and CuL_{aq}^- species.

The only apparent abnormality in this series is the formation of the 2-to-1 tryptophan-copper(II) complex. The values for log K_2 and ΔH_2 indicate that a more stable complex is being formed with tryptophan than with the other members of the series. (log K_2 is ~0.6 unit higher and ΔH_2 is ~1.3 kcal. per mole higher than the other members of the series, while log K_1 and ΔH_1 are comparable.) A model of this complex shows that a very symmetrical species can be formed with the planar indole groups comfortably in the trans position. Direct participation by the indole nitrogen or π bonding with the fused aromatic system of the indole groups may be taking place. This latter type of bond has been proposed by Wahlborg and Frieden (*22*) for the copper(II)-thyroxine complex.

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