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Formation Constant, Enthalpy, and Entropy Values for the Association of Alanine with H^+ and Cu^{2+} at 10, 25, and 40° [1a]

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Enthalpy changes for the protonation of alanate ion, A^-, and for the stepwise formation of CuA_2(aq) from Cu^{2+} and A^- have been determined calorimetrically at 10, 25, and 40°. The enthalpy changes were determined at ionic strength values of about 0.01 for alanine and 0.02 for the copper–alanine complexes. Thermodynamic equilibrium constants for these ion association reactions were determined at 10, 25, and 40°. A comparison is made of temperature coefficient and calorimetric methods for the determination of enthalpy changes. $\Delta S°$ values are calculated at each temperature.

Introduction

The present study was undertaken as part of a program to obtain precise calorimetric data for metal ion–amino acid interaction. Of particular interest is the determination of ΔH as a function of temperature and the comparison of the calorimetric ΔH values with those determined previously[2] by the temperature-coefficient method. In addition, the 25° data obtained in the present study extend a previous calorimetric study[3] of Cu^{2+}–glycine, –α-aminoisobutyric acid, –threonine, and –sarcosine interaction.

Few calorimetric data have been reported for metal ion–amino acid chelation, and, in the case of the copper–alanine system, no previous calorimetric studies have appeared in the literature. The temperature-coefficient method for the determination of enthalpies of ion association gives accurate values only in those instances where ΔH is not a function of temperature and equilibrium constants are known very accurately. A case in point has been discussed by Izatt, *et al.*[3]

This paper reports equilibrium constants and heats of formation of HA (alanine) from A^- and H^+ and of CuA^+ and CuA_2 from Cu^{2+} and A^- in dilute aqueous solution at 10, 25, and 40°. Results obtained by the temperature-coefficient method by others as well as in this study are compared with those determined calorimetrically.

Experimental Section

Materials.—A stock copper(II) perchlorate solution was prepared by dissolving C.P. CuO wire in a concentrated $HClO_4$–HNO_3 mixture, precipitating $Cu(OH)_2$ with a NaOH solution, washing the precipitate with water, and dissolving it in $HClO_4$ of known concentration. The solution was standardized with respect to Cu^{2+} by conventional analytical procedures. Stock ligand solutions were prepared by dissolving weighed quantities of C.P. alanine in water. The purity of the alanine was checked by infrared spectrophotometry.

Alanine solutions of various concentrations were titrated with standard $HClO_4$ to determine the optimum concentrations to be used in the calorimetric determinations. Solutions were then prepared by adding standard NaOH to measured portions of the stock alanine solution.

Equilibrium Constants.—Values for the thermodynamic equilibrium constants K_{D_1}, K_{D_2}, K_1, and K_2 for the reactions represented by eq. 1, 2, 3, and 4, respectively, wherein A^- represents

$$H_2A^+ \rightleftharpoons HA + H^+ \quad (1)$$

$$HA \rightleftharpoons H^+ + A^- \quad (2)$$

$$Cu^{2+} + A^- \rightleftharpoons CuA^+ \quad (3)$$

$$CuA^+ + A^- \rightleftharpoons CuA_2 \quad (4)$$

alanate ion, were determined as described previously.[2] Ionic strength, μ, and activity coefficient, γ, data tabulated by Crouthamel and Martin[4] at 25° and by Harned and Owen[5] at 10, 25, and 40° were used to determine γ values at specific μ values. The γ values for singly charged ions, γ_1, are tabulated as a function of $\mu^{1/2}$ at 10, 25, and 40°, and those values for doubly charged ions, γ_2, are tabulated *vs.* $\mu^{1/2}$ at 25°. Since the difference, $\gamma_1^4 - \gamma_2$, is small (<0.01 unit for $\mu < 0.02$) at all tabulated values of μ, the assumption was made that these differences at 10 and 40° would be about the same as at 25° in order to arrive at reasonable values for γ_2 at 10 and 40° from tabulated γ_1 values at those temperatures. The γ value for the zwitterion was taken as unity, in agreement with the experimental results of Smith and Smith.[6]

The Calorimeter.—A submarine isothermal calorimeter operating in a well-stirred, constant temperature water bath controlled to ±0.005° at 10, 25, and 40° was used. The calorimeter was a silvered dewar flask of about 150-ml. capacity. The mixing device was a modification of that used by Konnecke, Steinert, and Leibnitz[7] wherein the solutions were separated by a mercury seal until the inner container, which also served as a stirrer, was raised about 1 cm.

In addition to the stirrer and mercury, the flask contained a 40-ohm heater and a bead thermistor (*ca.* 75,000 ohms at 25°). The heating element consisted of about 2 ft. of enameled resistance wire wound upon a glass rod which had been permanently affixed horizontally to the inner wall of the calorimeter flask. The thermistor and heater were cemented in place with several coats of Tygon.

The thermistor constituted one arm of a Wheatstone bridge with precision resistance boxes serving as the other three arms. Bridge imbalance was recorded as a function of time. Temperature differences of 0.0002° were detectable. Direct current for both the Wheatstone bridge circuit and the calorimeter was supplied by a Nylab voltage stabilizer (New York Laboratory Supply Co., Inc.) which provided 6-v. d.c. stabilized to ±1%.

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[1] (a) Supported in part by Public Health Research Grant AM-02170, from the National Institute of Arthritis and Metabolic Diseases. (b) To whom inquirees concerning this article should be sent.

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[6] E. R. B. Smith and P. K. Smith, *J. Biol. Chem.*, **121**, 607 (1937).

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A Ratheon voltage stabilizer fed line voltage stabilized to ±1% into the Nylab stabilizer. Time intervals during electrical calibration experiments were measured by means of a stopwatch. A Type K-3 potentiometer (Leeds and Northrup) and a high sensitivity box galvanometer (Rubicon) were used to measure voltage differences during electrical calibration. All electrical leads were shielded and the shielding was grounded. A ballast heater was a part of the direct circuit at all times except during calibration runs to avoid current surges and maintain constant voltage output. An NBS certified 1-ohm resistor was used to measure amperage during calibration.

Calorimetric Determinations.—A complete determination consisted of the chemical run and two electrical calibrations runs, one preceding and one following the chemical run. A calibration run consisted of a rating period of about 12 min., a 2-min. period of heat input, and a second rating period of about 12 min. Temperature changes were essentially complete for a chemical run in less than 1 min. with the major temperature change occurring during the first few seconds after the solutions were mixed. All solutions were dilute and temperature changes were small; therefore, the heat capacity of the calorimeter and its contents changed very little when the reactant solutions were mixed. The occasional poor determination was eliminated from consideration by accepting as valid only those determinations for which the results of the two calibration runs agreed to within less than 1%.

The heat of ionization of water determined from the neutralization of perchloric acid by sodium hydroxide at ionic strengths of 0.02 and 0.01 was measured at 10, 25, and 40° as a confirmatory check on the electrical calibration. The heat of ionization of water, determined from the neutralization of dilute aqueous solutions of strong acids and bases, has been the subject of much investigation. The most recent determinations report the standard heat of ionization of water at 25° and $\mu = 0$ to be 13.334[8] and 13.337[9] kcal./mole. The $\Delta H°$ value for the heat of ionization of water was determined in this study as 13.90, 13.33, and 12.47 kcal./mole at 10, 25, and 40°, respectively. The uncertainties in these measurements are ±70 cal./mole at 10 and 25° and ±100 cal./mole at 40°. These results are somewhat lower than the heats of ionization at these three temperatures calculated from the ion product data of Harned and Hamer[10] and Harned and Owen[11]; however, the 25° value is in excellent agreement with the recent calorimetric determination.[8,9] The measured ΔH values for the association of H^+ and OH^- ($\mu \neq 0$) were used to calculate the contribution of this reaction to the heat measured for each calorimetric run.

A Beckman Model GS battery-operated pH meter was used to determine hydrogen ion activities. Buffer solutions were prepared as discussed by Bates.[12]

Three types of calorimetric determinations were made. The heat of protonation of A^- at μ values near 0.01 (type x runs) was calculated from the heats evolved when alkaline solutions of alanine were mixed with solutions of perchloric acid. The heat of formation of CuA_2 from Cu^{2+} and A^- (type y runs) was calculated from the heat evolved when alkaline solutions of alanine were mixed with copper(II) perchlorate solutions to produce solutions with 2:1 alanine–copper formality ratios. The heat of reaction of Cu^{2+} with A^- to form CuA^+ was calculated from type z runs. These runs differed from y runs in that the alanine–copper formality ratios were 1:1. The heat produced by the formation of CuA^+ was negligible in type y runs because the concentrations of CuA^+ were negligibly small for these runs.

A number of calorimetric determinations were made to measure thermal effects accompanying dilution of the reactants. In no case were thermal effects detectable.

A number of type x runs were performed at 25° using extremely basic alanine solutions. Although the results of these determinations showed the expected increase in average deviation from the mean because of the relatively large amounts of heat produced by the water formed, their mean was within a few hundredths of a kilocalorie of the result obtained at lower initial pH values.

Calculations.—The equations for the equilibria represented by the constants K_{D_1}, K_{D_2}, K_1, and K_2 can be combined with the mass balance equations for total alanine distributed among the five species A^-, HA, H_2A^+, MA^+, and MA_2 and for total copper distributed among the species Cu^{2+}, CuA^+, and CuA_2 to produce the equation

$$\begin{aligned}([MA^+]^3)(4K_2^2\gamma_1^4 - K_1K_2\gamma_2\gamma_1^2) &+ \\ ([MA^+]^2)(4K_2\gamma_1^2n - K_1\gamma_2 n + 2K_1K_2\gamma_2\gamma_1^2M_T) &+ \\ ([MA^+])(n^2 + K_1\gamma_2 n(A_T + M_T) + K_1K_2\gamma_2\gamma_1^2A_T &\times \\ (A_T - 2M_T)) - K_1\gamma_2A_TM_Tn &= 0 \end{aligned} \quad (5)$$

where M_T and A_T represent the formalities of the metal and alanine, respectively, the concentration of MA^+ is a function of known or calculable quantities if the activity of the hydrogen ion in the solution is known, and the quantity n is defined by the equation

$$n = 1 + \frac{\gamma_1 a_{H^+}}{K_{D_2}} + \frac{(a_{H^+})^2}{K_{D_1}K_{D_2}} \quad (6)$$

Equations representing γ_1 and γ_2 as functions of the concentrations of the ions present were obtained from a least-squares treatment of the tabulated γ and $\mu^{1/2}$ data. Tablulated γ values[4,5] were reproduced by these equations to ±0.001 unit. An approximate μ value was used to calculate values for use in eq. 5. Concentrations of all species were then calculated and a new μ value obtained. These calculations were repeated until successive γ values agreed within ±0.0001 unit.

$$\gamma_1\ (10°) = 1.000 - 1.1176\mu^{1/2} + 2.0975\mu - 1.8405\mu^{3/2} \quad (7)$$

$$\gamma_1\ (25°) = 0.9997 - 1.1680\mu^{1/2} + 2.4038\mu - 2.8390\mu^{3/2} \quad (8)$$

$$\gamma_1\ (40°) = 1.0001 - 1.2028\mu^{1/2} + 2.4092\mu - 2.3830\mu^{3/2} \quad (9)$$

$$\gamma_2\ (10°)\ 1.000 - 4.585\mu^{1/2} + 14.698\mu - 21.480\mu^{3/2} \quad (10)$$

$$\gamma_2\ (25°) = 0.9992 - 4.7670\mu^{1/2} + 15.9058\mu - 25.0727\mu^{3/2} \quad (11)$$

$$\gamma_2\ (40°) = 1.0002 - 4.8483\mu^{1/2} + 15.9106\mu - 23.6282\mu^{3/2} \quad (12)$$

Once the concentrations of the species in solution were known it was possible to calculate the desired heats of formation. Equation 13 represents the contributions of the various ion-association reactions to Q_m, the heat measured in a given calorimetric determination.

$$Q_m = R + [([HA])V_f - T]Q_{HA} + ([MA_2])V_fQ_{MA_2} + ([MA^+])V_fQ_{MA^+} \quad (13)$$

Where R represents the heat produced by the association of H^+ and OH^- to form H_2O when the ligand and the metal (or acid for type x runs) solutions were mixed, T represents the number of moles of zwitterion, HA, present in the initial alanine solution, V_f represents the volume of the final mixed solution, the bracketed quantities are the molar concentrations of the indicated species in the final mixed solution, each Q represents the heat produced when 1 mole of the subscript species is formed from its ions, and Q_m is the sum of the first two terms for type x runs, the first three terms for type y runs, and all terms for type z runs.

All calculations were programmed for the IBM 7040 computer.

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TABLE I

VALUES OF pK_{D_1}, pK_{D_2}, LOG K_1, AND LOG K_2 VALID AT $\mu = 0$ AND t = 10, 25, AND 40° FOR THE INTERACTION OF A^- WITH H^+ AND Cu^{+2}

Temp., °C.	pK_{D_1}	pK_{D_2}	log K_1	log K_2
0	2.43[a]	10.57[a]	8.93[a]	7.37[a]
			8.96[a]	7.29[a]
10	2.40	10.29	8.70	7.26
	2.39[a]	10.30[a]	8.77[a]	7.12[a]
			8.75[a]	7.13[a]
20	2.37[a]	10.04[a]	8.68[a]	7.00[a]
			8.63[a]	7.04[a]
25	2.37	9.89	8.54	6.98
	2.22[b]	9.97[b]		
	2.34[c]	9.87[c]	8.51[c]	6.86[c]
	2.35[d]	9.87[d]	8.40[d]	7.36[d]
	2.35[e]	9.83[e]		
30	2.36[a]	9.78[a]	8.58[a]	6.86[a]
			8.53[a]	6.93[a]
40	2.35	9.51	8.32	6.76
	2.36[a]	9.48[a]	8.39[a]	6.68[a]
			8.29[a]	6.69[a]
			8.24[a]	6.63[a]

[a] Results of Izatt, *et al.*[2] [b] A. Albert, *Biochem. J.*, **47**, 531 (1950). [c] C. B. Monk, *Trans. Faraday Soc.*, **47**, 292 (1951). [d] R. M. Keefer, *J. Am. Chem. Soc.*, **70**, 476 (1948). [e] P. K. Smith, A. C. Taylor, and E. R. B. Smith, *J. Biol. Chem.*, **122**, 109 (1937).

Results

Values of pK_{D_1}, pK_{D_2}, log K_1, and log K_2 are tabulated in Table I together with comparison data from the literature. Each value was obtained from at least three separate titrations conducted under a nitrogen atmosphere. Each titration consisted of at least ten points for which equilibrium constants could be calculated.

Discussion

The inadequacy of the temperature-coefficient method as applied to the determination of the enthalpy of formation of alanine and of its copper chelates is demonstrated. As seen in Table III, $\Delta H°$ is not independent of temperature even over relatively short temperature ranges, nor is it a simple function of temperature.

The $\Delta H°$ values in this study for Cu^{2+}–A^- interaction are similar to those reported by Izatt, *et al.*,[3] for the Cu^{2+}–glycine, –α-aminoisobutyric acid, –sarcosine, and –threonine systems.[3] In both studies the $\Delta H_2°$ values at 25° are significantly more negative than the corresponding $\Delta H_1°$ values, probably reflecting that more energy is required to dehydrate Cu^{2+} in the first

TABLE II

CALORIMETRIC DATA FOR TYPE x, y, AND z RUNS AT 10, 25, AND 40°[a–d]

Type x Runs. Reaction: $H^+ + A^- = HA$

t, °C.	μ	No. of runs	V_A, ml.	V_{acid}, ml.	F_A	F_{acid}	F_{NaA}	pH_A	pH_f	Q_m, cal.	$\Delta H°$, kcal./mole
10	0.0096	4	99.64	5.19	0.00906	0.1892	0.01018	11.27	6.06	10.53	−11.78
	0.0114	2	99.64	5.19	0.00906	0.1892	0.01018	11.53	6.77	11.43	−11.56
	0.0083	2	103.68	9.97	0.00999	0.0964	0.00939	11.01	7.29	10.70	−11.60
											−11.68 ± 0.10
25	0.0096	5	99.90	5.20	0.00903	0.1886	0.01014	10.71	6.06	9.76	−11.35
	0.0085	2	104.94	5.02	0.00994	0.0961	0.00922	10.63	6.78	10.79	−11.31
	0.0085	6	104.94	10.00	0.00994	0.0962	0.00922	10.63	6.62	10.73	−11.28
	0.0085	2	104.99	9.99	0.00994	0.0961	0.00922	10.61	6.46	10.64	−11.28
											−11.30 ± 0.11
40	0.0098	4	99.83	5.22	0.00899	0.1878	0.01010	10.51	6.28	9.67	−10.26 ± 0.06

Type y Runs. Reaction: $Cu^{2+} + 2A^- = CuA_2$

t, °C.	μ	No. of runs	V_A, ml.	V_M, ml.	F_A	F_M	F_{NaA}	pH_A	pH_f	Q_m, cal.	$\Delta H°$, kcal./mole
10	0.0192	5	99.64	8.48	0.01810	0.1043	0.02036	11.77	11.15	11.03	−10.85 ± 0.25
25	0.0185	5	99.90	8.90	0.01805	0.1000	0.02029	11.10	10.05	9.50	− 9.75 ± 0.07
40	0.0190	5	99.83	8.50	0.01797	0.1036	0.02020	10.65	9.65	9.06	− 9.64 ± 0.05

Type z Runs. Reaction: $Cu^{2+} + A^- = CuA^+$

t, °C.	μ	No. of runs	V_A, ml.	V_M, ml.	F_A	F_M	F_{NaA}	pH_A	pH_f	Q_m, cal.	$\Delta H°$, kcal./mole
10	0.0190	5	99.64	8.48	0.00906	0.1043	0.01018	11.53	6.58	5.87	5.38 ± 0.17
25	0.0173	5	99.27	8.20	0.00903	0.1045	0.00799	10.56	5.15	3.70	4.51
	0.0167	1	99.27	7.82	0.00903	0.1045	0.00799	10.56	5.24	3.67	4.50
											4.50 ± 0.10
40	0.0190	5	99.83	8.53	0.00899	0.1036	0.01010	10.54	6.29	4.45	−3.99 ± 0.09

[a] Q_m represents the total measured heat. The subscripts A and f refer to the initial alanine solutions and the final mixed solutions, respectively. F_{NaA}, F_A, F_{acid}, and F_M refer to the formalities of sodium ion and alanine in the initial alanine solution, acid in the initial acid solution, and Cu^{2+} ion in the initial metal ion solution, respectively. [b] The bath temperature for these runs was 39.4 rather than 40°. All contants were evaluated at 39.4° as well as at 40°; the former were used in these calculations while the latter are tabulated in Table I. [c] The ratios of the concentrations of CuA_2 to CuA^+ were 4100:1, 1000:1, and 830:1 for y runs at 10, 25, and 40°, respectively, and 1:4.4, 1:6.7, and 1:5.1 for z runs at the three temperatures. Uncertainties of the $\Delta H°$ values are given as the average deviation from the mean and do not include the contributions of uncertainties in pK_D values of ±0.01 unit and in log K values of ±0.03 unit. [d] A summary of the $\Delta H°$ and $\Delta S°$ values obtained in the present study together with previous results is given in Table III.

TABLE III

STANDARD ENTHALPY AND ENTROPY CHANGE VALUES FOR THE STEPWISE FORMATION OF H_2A^+ AND CuA_2 FROM THEIR RESPECTIVE IONS

	$H^+ + A^- = HA$		$HA + H^+ = H_2A^+$		$Cu^{2+} + A^- = CuA^+$		$CuA^+ + A^- = CuA_2$	
Temp., °C.	$\Delta H°$, kcal./mole	$\Delta S°$, e.u.	$\Delta H°$, kcal./mole	$\Delta S°$, e.u.	$\Delta H°$, kcal./mole	$\Delta S°$, e.u.	$\Delta H°$, kcal./mole	$\Delta S°$, e.u.
10	−11.68	5.8			−5.38	20.8	−5.47	13.9
25	−11.30	7.4			−4.51	23.9	−5.24	14.3
	−10.8[a]		−0.6[a]					
	−11.0[b]		−0.8[b]					
40	−10.26	10.8			−3.99	25.3	−5.65	12.9
10–40	−10.4[c]	10.3[c]	−0.7[c]		−5.1[c]	21.8	−6.8[c]	9.0[c]
0–40	−10.6[d]	9.8[d]	−0.7[d]		−5.6[d]	20.7	−6.2[d]	10.7[d]

[a] Calorimetric results of J. M. Sturtevant, *J. Am. Chem. Soc.*, **64,** 726 (1942). [b] Results of Smith, *et al.*, see footnote *e* to Table II. [c,d] Results calculated by the temperature-coefficient method in this research and by Izatt, *et al.*,[2] respectively.

step than CuA^+ in the second step. It is significant that changes from one α-amino acid to another as the chelating group is changed do not affect to any large extent the observed $\Delta H_i°$ values and that the $\Delta H_1°$ and $\Delta H_2°$ values are nearly equal in all cases studied. The significantly lower value of K_2 compared to K_1 in the case of the Cu^{2+}–A^- system appears to be primarily a result of the markedly lower value of $\Delta S_2°$ compared to $\Delta S_1°$. This effect was also observed in the previous study,[3] the values of $\Delta S_2°$ and $\Delta S_1°$ in both studies being comparable in magnitude.

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Proton Nuclear Magnetic Resonance Studies of Metal Gluconate Complexes. I. Lead(II) and Bismuth(III)

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Proton n.m.r. spectra have been recorded for the complexes formed by D-gluconic acid with Mg(II), Al(III), Zn(II), Mo(VI), Pb(II), and Bi(III) ions under alkaline conditions. Only the Pb(II) and Bi(III) complexes exhibit chemical shifts for the C–H protons of the gluconate anion that are significantly different from those observed for the free ligand. Mole ratio studies based on chemical shift changes establish that four metal gluconate species are formed with the Pb(II) and Bi(III) ions under alkaline conditions: (a) $Pb:GH_4 = 3:2$; (b) $Pb:GH_4 = 2:1$, (c) $Bi:GH_4 = 1:2$, and (d) $Bi:GH_4 = 1:1$. Formulas and structures are proposed for these four species on the basis of n.m.r. data and acid–base titrations. The data support the conclusion that the carboxylate group and the α, β, and γ alcoholic oxygens of the gluconate anion are the only donor groups involved in coordination.

Although the complexes formed between metal ions and D-gluconic acid have been extensively studied,[1] the nature of the metal–ligand bonding and the structures of the complexed species have not been well established. Proton nuclear magnetic resonance spectroscopy has been shown to be useful for structural and bonding studies of metal–EDTA complexes.[2,3] More recently the results of an n.m.r. study of gluconic acid, its salt, and its lactones as well as of other related hydroxy acids have been presented.[4] A brief study of the lead(II) and bismuth(III) tartrate complexes by n.m.r. has indicated the value of such studies for determining the bonding and formulas of hydroxy acid complexes.[5] On the basis of these latter two investigations, proton n.m.r. studies of metal gluconate complexes should be expected to provide a better understanding of the formulas, bonding, and the structures of the complex species. The present discussion summarizes the results of such a study for several metal gluconate complexes in aqueous solution.

Experimental Section

The proton n.m.r. spectra were recorded with a Varian A-60 high-resolution spectrometer equipped with a 60-Mc. oscillator. Chemical shifts were measured using tetramethylammonium chloride (TMA) as an internal reference. The resonance for this material is 3.177 p.p.m. downfield from 3(trimethyl silyl)1-propanesulfonic acid, sodium salt (TMS*), and the reported

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