The Thermodynamics of Ionization of Amino Acids. I. The Ionization Constants of γ-Aminobutyric Acid

By Edward J. King

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The electromotive forces of cells with hydrogen and silver–silver chloride electrodes which contained buffer mixtures either of γ-aminobutyric acid and hydrochloric acid or of the amino acid, its sodium salt, and sodium chloride were measured at 5° intervals from 10 to 50°. From these data the thermodynamic ionization constants, K_1 for the ionization of the carboxyl group and K_2 for the ionization of the ammonium group, were calculated. The changes in free energy, enthalpy, entropy and heat capacity associated with the ionization in the standard state can be obtained from the equations $-\log K_1 = (1209.07/T) - 3.7820 + 0.012605T$ and $-\log K_2 = (2804.84/T) + 0.5879 + 0.0018797T$.
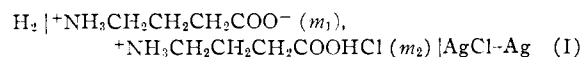
The ionization constants and related thermodynamic properties of twenty amino acids and one peptide are available from precise electromotive force measurements by the method of Harned and Owen.[1] The accumulation of more data of this nature is desirable in order to furnish a broad basis for the comprehensive examination of the thermodynamic properties associated with ionization. A comparison of the existing data for amino acids with that for substituted ammonium ions and fatty acids offers the possibility of new insight into the phenomenon of ionization. This will be dealt with in a subsequent communication.

The thermodynamic ionization constants of three ω-amino acids have been reported over a range of temperatures: glycine,[2,3] β-alanine[4] and ε-aminocaproic acid.[5] Because of the wide divergence in behavior of the last two of these acids it is of interest to determine the thermodynamic properties of the ionization of γ-aminobutyric acid which follows β-alanine in the series.
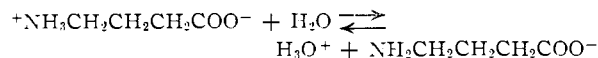
All of these ω-amino acids exist predominantly in the dipolar ion form in aqueous solution. Their dielectric increments, for example, increase progressively with chain length, an indication of increasing separation of the $-NH_3^+$ and $-COO^-$ groups.[6] In the case of γ-aminobutyric acid there are about 300,000 times as many dipolar ions, $^+NH_3CH_2CH_2CH_2COO^-$, as neutral molecules, $NH_2CH_2CH_2CH_2COOH$, in aqueous solution.[7] The first ionization constant, K_1, which corresponds to the reaction represented by
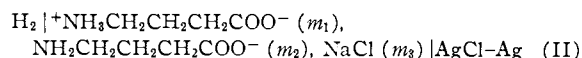
$$^+NH_3CH_2CH_2CH_2COOH + H_2O \rightleftharpoons$$
$$H_3O^+ + {}^+NH_3CH_2CH_2CH_2COO^-$$
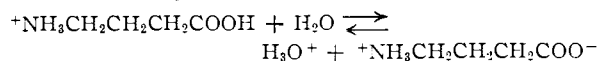
has been obtained from measurements on the cell

$$H_2 \,|\, ^+NH_3CH_2CH_2CH_2COO^- \,(m_1),$$
$$^+NH_3CH_2CH_2CH_2COOHCl \,(m_2) \,|AgCl\text{-}Ag \quad (I)$$

The second ionization constant, K_2, for the reaction represented by

$$^+NH_3CH_2CH_2CH_2COO^- + H_2O \rightleftharpoons$$
$$H_3O^+ + NH_2CH_2CH_2CH_2COO^-$$

was derived from data on the cell

$$H_2 \,|\, ^+NH_3CH_2CH_2CH_2COO^- \,(m_1),$$
$$NH_2CH_2CH_2CH_2COO^- \,(m_2), \; NaCl \,(m_3) \,|AgCl\text{-}Ag \quad (II)$$

Measurements were made at 5° intervals from 10 to 50° so that the related thermodynamic properties could be calculated.

Experimental

Potassium phthalimide and γ-chlorobutyronitrile from Distillation Products Industries were used as starting materials for the synthesis of γ-aminobutyric acid.[8] The crude acid was purified by repeated reprecipitation from concentrated aqueous solution with redistilled absolute ethanol. The final product was dried first at 45–50° overnight[9] and finally in a vacuum desiccator. It was found by formol titration to be 99.5 ± 0.2% pure. It has been assumed in calculating the ionization constants that the 0.5% impurity is inert. There was less than 0.2% water as determined by the Karl Fischer method.[10] Semi-quantitative tests[11] showed less than 0.004% chloride, iron, phosphate, heavy metals or ammonia.[12]

Anal. Calcd. for $C_4H_9NO_2$: C, 46.59; H, 8.80; N, 13.58. Found: C, 46.6₀; H, 8.7₃; N, 13.3₅.[13]

Standard hydrochloric acid was prepared by dilution of the constant boiling acid and its composition was checked by gravimetric analysis to within 0.06%. The sodium hydroxide solution was prepared by diluting a clear 50% solution with carbon dioxide-free water. It gave no turbidity with barium chloride. The base was standardized against both the standard acid and potassium acid phthalate (National Bureau of Standards Sample 84c); the two standardizations agreed within 0.05%. The sodium chloride has been described before.[3]

The apparatus and general techniques were substantially the same as in earlier work.[3,14,15] To conserve materials a single cell was filled with each buffer solution. Duplicate pairs of electrodes were used in each cell. The standard deviation between duplicate pairs was ±0.024 mv. for the whole set of measurements. Measurements over the temperature range were made as previously described[14] except that for cell II the measurements were made from 25 to 50° on the first day and at 25° and below on the day following. Agreement between successive readings at 25°, three or four in number, was used as a criterion of the performance of the cells. The standard deviations between initial and final readings at 25° were ±0.048 mv. for cell I and ±0.29 mv. for cell II. The poorer performance of cell II is most prob-

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(7) Reference 6, p. 99.

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(10) K. Fischer, *Angew. Chem.*, 48, 394 (1935).

(11) M. P. Stoddard and M. S. Dunn, *J. Biol. Chem.*, 142, 329 (1942).

(12) The white precipitate which forms upon addition of Nessler reagent to a solution of the amino acid must be removed by centrifugation and more reagent added in order to detect as little as 0.004% ammonia.

(13) Analyses by Schwarzkopf Microanalytical Laboratory, Middle Village 79, N. Y. The nitrogen was determined by the Dumas method.

(14) E. J. King and G. W. King, THIS JOURNAL, 74, 1212 (1952).

(15) E. J. King, *ibid.*, 75, 2204 (1953).

ably a consequence of the increased solubility of silver chloride in the alkaline buffer solutions. The major part of the decrease in electromotive force occurred after the cells had stood overnight. This has been corrected for by adjusting the inital readings at 25° and those which followed them on the second day to correspond with those on the first day.

The electromotive forces, corrected to a hydrogen pressure of one atmosphere, can be represented as a function of temperature by the equation

$$E_1 = E_{25} + a(t - 25) + b(t - 25)^2 \qquad (1)$$

The parameters of this equation for electromotive forces in absolute volts and concentrations in moles per kg. of water are given in Table I. All weighings have been corrected for the buoyancy of air. The standard deviations between the observed electromotive forces and those calculated from equation 1 are ±0.060 mv. for cell I and ±0.028 mv. for cell II.

TABLE I

PARAMETERS OF EQUATION 1

$m_1 = m_2$	E_{25}	$a \times 10^4$	$b \times 10^7$
		Cell I	
0.00999	0.58516	5.25	−9.2
.01502	.57557	4.91	−5.8
.01756[a]	.57204	4.79	−6.5
.02019	.56882	4.66	−7.5
.02458[a]	.56439	4.55	−6.5
.02997	.55990	4.37	−6.8
.04020	.55342	4.17	−8.0
.04970	.54854	3.99	−7.8

m_1	m_2/m_1	m_3/m_1	E_{25}	$a \times 10^5$	$b \times 10^6$
			Cell II		
0.02192	0.5062	0.5021	0.94555	2.00	−2.70
.02617	.5062	.5021	.94123	0.40	−2.66
.03025	.5062	.5021	.93779	−0.50	−2.70
.03266	.5063	.5021	.93601	−1.10	−2.78
.04250	.5063	.5021	.92963	−3.10	−2.62
.04821	.5063	.5021	.92668	−4.30	−2.75

[a] Measurements were omitted at 10°.

Calculations and Results

The thermodynamic ionization constants were obtained from the intercepts at zero ionic strength (μ) of plots of the functions pK'_1 and pK'_2 defined by the familiar relations[1]

$$pK'_1 = - \log [(m_1 + m'_H)m_H/(m_2 - m'_H)] \qquad (2)$$

$$pK'_2 = (\mathfrak{F}/2.3026RT)(E_{II} - E^0) + \log [m_3(m_1 + m'_{OH})/(m_2 - m'_{OH})] \qquad (3)$$

The apparent hydrogen and hydroxyl ion concentrations were calculated from the electromotive force data by

$$- \log m'_H = (\mathfrak{F}/2.3026RT)(E_I - E^0) + [2S\sqrt{\mu d_0}/(1 + A d \sqrt{\mu d_0})] + \log m_2 \qquad (4)$$

$$- \log m'_{OH} = (\mathfrak{F}/2.3026RT)(E_{II} - E^0) + \log m_3 - \log K_w \qquad (5)$$

The latest values of the physical constants were used.[16] In the second term on the right-hand side of equation 4, which is the Debye–Hückel approximation for the activity coefficient of hydrochloric acid in the buffer solution, the value of the parameter d was chosen as 3.85 Å. to give extrapolations with low slopes. This choice of d does not affect the final value of the ionizaton constant.[1,14] The negative logarithms of the ionization constants ob-

tained from these calculations are given in Table II. The standard deviations of the extrapolated values are ±0.0009₁ for pK_1 and ±0.0011₁ for pK_2, the equivalent of ±0.054 and ±0.065 mv., respectively, in the electromotive forces. No values of the thermodynamic constants are recorded in the literature. Ley,[17] on the basis of conductance measurements, reported apparent ionization constants of 5.9×10^{-5} and 3.7×10^{-11}. These are in fair agreement with the values 9.31×10^{-5} and 2.78×10^{-11} at 25° found in this investigation.

TABLE II

THE NEGATIVE LOGARITHMS OF THE IONIZATION CONSTANTS OF γ-AMINOBUTYRIC ACID

Temp., °C.	pK_1	pK_2
10	4.0571 (0)	11.0260 (3)
15	4.0459 (0)	10.8634 (2)
20	4.0380 (6)	10.7057 (−9)
25	4.0312 (−1)	10.5557 (2)
30	4.0266 (−8)	10.4091 (−7)
35	4.0251 (−6)	10.2694 (3)
40	4.0272 (11)	10.1336 (5)
45	4.0293 (8)	10.0025 (7)
50	4.0320 (−7)	9.8740 (−8)

The variation of the ionization constants with temperature can be expressed by the following equations[18]

$$pK_1 = (1209.07/T) - 3.7820 + 0.012605T \qquad (6)$$

$$pK_2 = (2804.84/T) + 0.5879 + 0.0018797T \qquad (7)$$

The number in parentheses following each experimental value of pK in Table II is the difference in the fourth decimal place between that value and the one calculated from equation 6 or 7. The changes in free energy (ΔF^0), enthalpy (ΔH^0), entropy (ΔS^0), and heat capacity (ΔC_p^0) associated with the ionization reactions in the standard state, as derived from equations 6 and 7 by thermodynamic methods,[18] have the following values at 25°:

	ΔF^0, cal. mole^{-1}	ΔH^0, cal. mole^{-1}	ΔS^0, cal. mole^{-1} deg.$^{-1}$	ΔC_p^0, cal. mole^{-1} deg.$^{-1}$
First ionization	5500	405	−17.0₉	−34
Second ionization	14401	12070	−7.8₂	−5

The minimum probable errors in these quantities are, respectively, ±2.1, ±16, ±0.06 and ±2 based on random errors of ±0.05 mv. in the electromotive forces and standard potentials and reasonable estimates of the weighing errors involved in making up the solutions. As a result of the lack of agreement above 25° between the standard potentials of Harned and Ehlers[19] and those of the author,[15] the values of the last three thermodynamic properties may be too low by 35, 0.12 and 1.8 units, respectively. The assumption that the 0.5% impurity is inert is another possible source of error. It is probable that the absolute errors in the thermodynamic properties are two to three times the minimum probable errors given above.

These data show that in all respects γ-aminobu-

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tyric acid occupies an intermediate position between β-alanine and ϵ-aminocaproic acid. The changes in enthalpy and entropy probably reflect the decrease in flexibility of the hydrocarbon chain with removal of the carboxyl hydrogen in the first ionization and the increase in flexibility resulting from removal of the proton from the ammonium group in the second ionization. Further discussion of these properties in relation to those of the other amino acids will be dealt with in another communication.

Spectrophotometric Studies of Dissociation Constants of Nitroguanidines, Triazoles and Tetrazoles

BY JOHN E. DEVRIES AND E. ST. CLAIR GANTZ

The dissociation constants of several derivatives of nitroguanidine and related compounds have been determined by spectrophotometric means. The method of Stenstrom and Goldsmith has been applied to compounds exhibiting weakly acidic (monoprotic and diprotic) and basic dissociation. Each pK value was obtained graphically from a smooth curve plotted from several absorption observations over the useful pH range using buffered solutions. Sodium chloride was added to maintain a constant ionic strength of one. Dissociation constants too low (pK 10 to 12) to be determined by potentiometric titration were easily measured. Where potentiometrically determined values were available for comparison, good agreement was obtained.

Ultraviolet absorption spectra have been reported for a large number of nitro-substituted guanidines and tetrazoles. In some cases absorption differences were noted when acidic *versus* basic solutions of these materials were measured.[1-3] No instances are reported where these differences were employed to arrive at a measure of the dissociation constants of these compounds.

The acidic properties of nitroguanidines and tetrazoles have been discussed and in cases where acid dissociation was extensive, potentiometric titration has been used to determine dissociation constants.[4-6] In cases where there was only slight dissociation, potentiometric methods were not feasible. The need for information concerning relative acidities of these weaker acids and bases was evident. The application of a spectrophotometric procedure provided a convenient method for determining dissociation constants for several nitroguanidines. A variety of nitramines has been included in this study to illustrate the applicability of the method.

Apparatus

The absorption spectra were measured using a Beckman model DU spectrophotometer. A Beckman model G, pH meter equipped with general purpose glass electrode was used for all pH measurements. Sodium ion corrections for the glass electrode were applied where necessary.

Experimental

The method of Stenstrom and Goldsmith[7] has been used by several workers in determining dissociation constants

of weak acids. The method has been extended to include two step ionization processes.[8,9]

The values for the ionization constants K_1 and K_2 are calculated as

$$pK_1 = pH - \log \frac{A_1 - A^\circ}{A' - A_1}$$

$$pK_2 = pH - \log \frac{A_2 - A'}{A'' - A_2}$$

where

A_1 = absorbance[10] of the mixture of the two species, un-ionized and singly ionized species

A° = absorbance of the un-ionized molecule

A' = absorbance of the singly ionized ion

A_2 = absorbance of the mixture of singly and doubly ionized species

A'' = absorbance of the doubly ionized ion

Absorbance values are used throughout the equations rather than molar absorptivity as in the original derivation[7,11] since cell length and molar concentration are constant for each series of measurements.

The use of these equations is simplified by plotting the relationship between pH and absorbance. At the midpoint of the break in the curve, $pH = pK$ since the log term equals zero.

In the present work, the absorption spectrum of each buffered solution was recorded in the ultraviolet range 200–400 mμ. Wave length peaks characteristic of the un-ionized molecule or ionized species were selected for use. In general, one major absorption peak was exhibited in the ultraviolet region by the compounds investigated and this peak most strongly illustrated the change in absorbance with change in pH of the medium. By employing a large number of buffered solutions over the pH range (1–14), it was possible to plot a smooth curve showing the relationship between pH and absorption. By careful measurement then the pK value could be easily obtained graphically.

In the case of the compounds for which the second dissociation constant was investigated, the removal of the second proton resulted in little or no shift in the main absorption peak.

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