[Contribution from the Chemical Laboratory of the University of California]

DISSOCIATION CONSTANTS AND HEATS OF IONIZATION OF SOME SIMPLE AMINO ACIDS AND PEPTIDES

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Introduction

Owing to their importance in biochemistry and in the theory of amphoterics, the acidic and basic dissociations of amino acids and peptides in aqueous solution have been measured by many investigators. The agreement among their results is not very good, and the values obtained by different investigators are not always comparable as the experiments have been carried out at different temperatures.

We have measured the acidic and basic dissociation constants of glycine, α-alanine, glycylglycine and glycylalanine in aqueous solution at two temperatures, 20 and 0°. Since the change of the logarithm of a dissociation constant with the reciprocal of the absolute temperature is given by the equation $d \ln K/d\,(1/T) = -\Delta H/R$, and since ΔH may be considered as approximately constant over small ranges of temperature, our measurements enable us to express our own values of the dissociation constants and those of other investigators at any one temperature in the neighborhood of room temperature. We have chosen 25° for this purpose. We have also calculated the heats of ionization of the substances acting as acids and bases from our data. Dernby[1] has measured the K_b of glycine and of glycylglycine at two temperatures. The heat of basic dissociation calculated from his results agrees very closely with ours in the case of glycine, but there is a difference of about a thousand calories in the case of glycylglycine. We also believe that calorimetric measurements have been made on some of the amino acids which would permit a calculation of the heats of ionization, but we have not been able to find the data.

Method

The electrometric method was used in determining the dissociation constants. This consists of measuring the P_H of solutions of the amphoteric mixed with half an equivalent of hydrochloric acid or of sodium hydroxide by means of a hydrogen electrode balanced against a normal calomel electrode.

Actually the P_H's of solutions of the amphoterics to which various quantities of hydrochloric acid or sodium hydroxide had been added were measured, and these values of the P_H were plotted against the number of equivalents of acid or base added. In the figure these titration curves

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[1] Dernby, *Compt. rend. trav. lab. Carlsberg*, **11**, 263 (1916).

are shown for the four amphoterics at 20°. The curves obtained at 0° are so similar to those at 20° that they have not been shown.

An acidic or basic dissociation constant can be calculated from any point on such curves. However, it is best to use points which represent the addition of half an equivalent of acid or base to the amphoteric.

At these half-way stages of the titrations the concentrations of the undissociated amphoteric and of its ion are, respectively, $^1/_2\ C\ -\ M$, in which C is the total molal concentration of the amphoteric in all its forms and M is the molality of the hydrogen or hydroxide ion. Hence

$$K_a = (\mathrm{H}^+) \times \left[\frac{1}{2}\,C - \frac{Kw}{(\mathrm{H}^+)}\right] \Big/ \left[\frac{1}{2}\,C + \frac{Kw}{(\mathrm{H}^+)}\right] \quad \text{and}$$

$$K_b = \frac{Kw}{(\mathrm{H}^+)} \times \left[\frac{1}{2}\,C - (\mathrm{H}^+)\right] \Big/ \left[\frac{1}{2}\,C + (\mathrm{H}^+)\right]$$

The first of these equations is practically equivalent to the equation $K_a = (\mathrm{H}^+)$, in the case of the substances used by us.

It may be noted that the equilibrium constants obtained in this way are of a hybrid character, for C is a true molal concentration while (H^+) as measured by the hydrogen electrode is an activity.

Results

The *P*H values at the half titration points and the corresponding acidic and basic dissociation constants are shown in Table I.

TABLE I

VALUES OF *P*H AT HALF TITRATION. AND DISSOCIATION CONSTANTS

Subs.	Temp., °C.	*P*H (base added)	K_a	*P*H (acid added)	K_b
Glycine	0	10.315	4.81×10^{-11}	2.575	2.34×10^{-13}
	20	9.724	1.875×10^{-10}	2.587	1.455×10^{-12}
α-Alanine	0	10.464	3.41×10^{-11}	2.575	2.34×10^{-13}
	20	9.825	1.485×10^{-10}	2.587	1.455×10^{-12}
Glycylglycine	0	8.796	1.6×10^{-9}	3.0925	1.18×10^{-12}
	20	8.254	5.57×10^{-9}	3.121	7.55×10^{-12}
Glycylalanine	0	8.963	1.09×10^{-9}	3.0925	1.18×10^{-12}
	20	8.356	4.41×10^{-9}	3.121	7.55×10^{-12}

These titrations were made by adding 0.1 *N* acid or base to 0.02 *N* amino acid or peptide, so that at the half-titration points the total concentration of the organic substance was 0.0182 *N*.

In all our calculations we have the following values for *Kw:* 1.005×10^{-14} at 25°, 0.676×10^{-14} at 20° and 0.114×10^{-14} at 0°. The first and last are those given by Lewis and Randall in their book, "Thermodynamics." The value at 20° was obtained from the other two by assuming that $\ln Kw$ is proportional to $1/T$ between 0 and 25°.

Both the ΔH's of dissociation and the equilibrium constants at 25° can be calculated from the results at 20 and 0° by means of the equation

$\ln K_2/K_1 = (-\Delta H/R) \times \Delta(1/T)$. The results of these calculations are shown in Table II.

TABLE II

HEATS OF DISSOCIATION AND EQUILIBRIUM CONSTANTS AT 25°

Substance	K_a	ΔH_a, cal.	K_b	ΔH_b, cal.
Glycine	2.54×10^{-10}	10,700	2.21×10^{-12}	14,600 (14,300)
α-Alanine	2.06×10^{-10}	11,450	2.21×10^{-12}	14,600
Glycylglycine	7.41×10^{-9}	9,830	1.15×10^{-11}	14,800 (16,000)
Glycylalanine	6.025×10^{-9}	11,100	1.15×10^{-11}	14,800

The figures in parentheses are those calculated from Dernby's data

In Table III we have collected most of the published values for the dissociation constants of the four amphoterics discussed in this article. Where necessary we have recalculated the published data so as to obtain the values corresponding to 25°, and to a value of $Kw_{25°} = 1.005 \times 10^{-14}$. In compiling this table we have used to a large extent a collection of the dissociation constant data for all the amino acids and peptides published by P. Kirk and C. Schmidt.[2]

The inner salt theory of amino acids as propounded by E. Q. Adams[3] and N. Bjerrum,[4] throws much light on the electrolytic dissociation of these substances and of the peptides. In this theory glycine is represented by the formula $H_3N^+CH_2COO^-$, and K_a and K_b are considered as hydrolysis constants. Two other constants are obtained by the equations $K^1_a = Kw/K_b$ and $K^1_b = Kw/K_a$. K^1_a and K^1_b are, respectively, the constants of the carboxylic acid, $H_3N^+CH_2COOH$, and of the amine, $H_2NCH_2COO^-$. Both Adams and Bjerrum point out that K^1_a is naturally much greater than K_a for acetic acid, owing to the repulsion of the hydrogen ion by the positive charge. The influence of the nitrogen apart from its positive charge may be assumed to be comparatively small on the basis of its position in the periodic table. In the same way K^1_b might be expected to be markedly greater than the K_b of $H_2NCH_2COOCH_3$.

Bjerrum has pointed out that in the peptides the distance of the positive charge from the carboxyl and that of the negative charge from the amino group is greater than in the amino acids, and that this would account for the fact that the values of K^1_a and K^1_b of the peptides are less than those of the amino acids. The work of Simms[5] confirms this, as he has found that K^1_a and K^1_b of glycylglycylglycine are less then the corresponding constants of glycylglycine.

It may be also noticed that the dissociation constants of the peptides indicate that the influence of a charged group on a dissociation of hydrogen ion is less affected by the intervening atoms than the corresponding

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[2] University of California, *Pub. Physiol.*, [6] **7**, 57–69 (1929).

[3] Adams, THIS JOURNAL, **38**, 1503 (1916).

[4] Bjerrum, *Z. physik. Chem.*, **104**, 147 (1923).

[5] Simms, *J. Gen. Physiol.*, **11**, 629 (1928).

TABLE III

COMPILATION OF PUBLISHED VALUES OF THE DISSOCIATION CONSTANTS AT 25°

K_a	K_b	Method	Author
		Glycine	
3.4×10^{-12}	2.9×10^{-12}	Conductivity and ester hydrolysis	Winkelblech[10]
[6]2.92×10^{-10}	[6]9.23×10^{-12}	Conductivity	Euler[11]
[6]1.85×10^{-10}	[6]2.52×10^{-12}	Electrometric	Michaelis and Rona[12]
[6]2.20×10^{-10}	[6]2.70×10^{-12}	Electrometric	Sörensen[13]
[6]1.62×10^{-10}	[7]2.44×10^{-12}	Electrometric	Dernby[14]
[6]2.22×10^{-10}	[6]2.70×10^{-12}	Electrometric	Hirsch[15]
1.80×10^{-10}	2.6×10^{-12}	Electrometric	Harris[16]
2.3×10^{-10}	2.14×10^{-12}	Electrometric	Levene, Simms and Pfaltz[17]
2.54×10^{-10}	2.21×10^{-12}	Electrometric	Branch and Miyamoto
		α-Alanine	
9.0×10^{-10}	3.8×10^{-12}	Conductivity and ester hydrolysis	Winkelblech[10]
2.3×10^{-10}	3.1×10^{-12}	Conductivity	Ley[18]
1.8×10^{-10}	2.5×10^{-12}	Electrometric	Harris[16]
[6]2.21×10^{-10}	[6]1.8×10^{-12}	Electrometric	Hirsch[15]
1.91×10^{-10}	2.24×10^{-12}	Electrometric	Simms[19]
2.06×10^{-10}	2.21×10^{-12}	Electrometric	Branch and Miyamoto
		Glycylglycine	
[8]6.98×10^{-9}	[8]1.47×10^{-11}	Electrometric	Eckweiler, Noyes and Falk[20]
2.73×10^{-8}	3.62×10^{-11}	Conductivity	Euler[11]
[6]4.89×10^{-9}	[7]1.45×10^{-11}	Electrometric	Dernby[14]
5.3×10^{-9}	1.4×10^{-11}	Electrometric	Harris[16]
[6]6.54×10^{-9}	[9]8.78×10^{-12}	Electrometric	Levene, Simms and Pfaltz[17]
7.41×10^{-9}	1.15×10^{-11}	Electrometric	Branch and Miyamoto
		Glycylalanine	
5.62×10^{-9}	1.41×10^{-11}	Electrometric	Simms[19]
6.025×10^{-9}	1.15×10^{-11}	Electrometric	Branch and Miyamoto

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[6] Calculated from data obtained at 18°.

[7] Recalculated from data so as to correspond with K_w 25° $= 1.005 \times 10^{-14}$.

[8] Calculated from results of an electrical titration published by the authors. The authors do not state the temperature, so we have assumed that the measurements were made at 20°.

[9] Calculated from data obtained at 30°.

[10] Winkelblech, *Z. physik. Chem.*, **36**, 546 (1901).

[11] Euler, *ibid.*, **51**, 213 (1907).

[12] Michaelis and Rona, *Biochem. Z.*, **49**, 232 (1913).

[13] Sörensen, *ibid.*, **21**, 131 (1909).

[14] Dernby, *Compt. rend. trav. lab. Carlsberg*, **11**, 263 (1916).

[15] Hirsch, *Biochem. Z.*, **147**, 433 (1924).

[16] Harris, *Proc. Roy. Soc. London*, **95B**, 440 (1923-1924).

[17] Levene, Simms and Pfaltz, *J. Biol. Chem.*, **61**, 445 (1924).

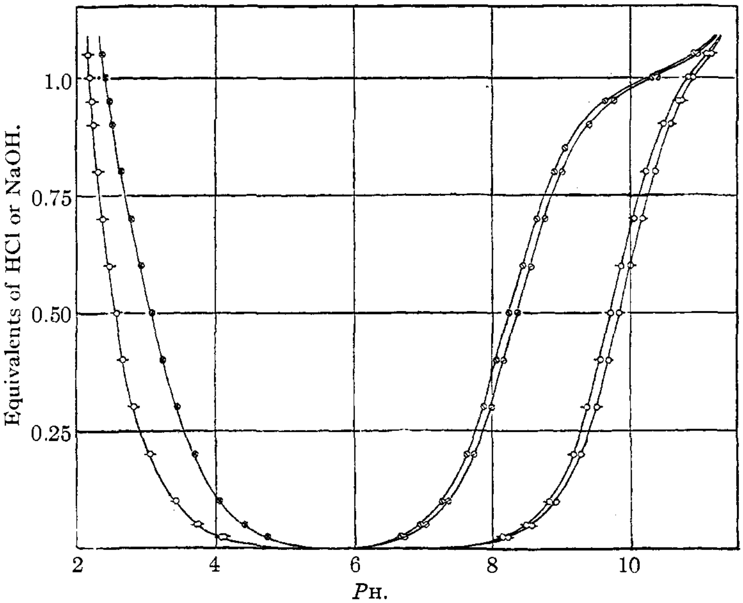
[18] Ley, *Ber.*, **42**, 354 (1909).

[19] Simms, *J. Gen. Physiol.*, **11**, 629 (1928).

[20] Eckweiler, Noyes and Falk, *ibid.*, **3**, 291 (1920-1921).

influence of electrically neutral groups. This may be shown by the following comparisons.

From K_a of acetic acid = $10^{-4.7}$ and K^1_a of glycine = $10^{-2.3}$, the Derrick factor[21] for the $-{}^{+}NH_3$ group substituted in the α-position may be found to be equal to 1.0. The factor for this group substituted in the δ-position can be obtained from K_a of $C_6H_5CONHCH_2COOH$ = $10^{-3.66}$ and K^1_a of glycylglycine = $10^{-3.06}$. It is equal to 0.2. In the case of electrically neutral groups these factors decrease to about one-third of their values for every intervening atom. Thus the factor for α-substituted chlorine is 0.68 and for the same halogen on the δ-atom it is 0.02.[22]



·O, Glycine; O·, α-alanine; ⦸, glycylglycine; ⊘, glycylalanine; ·O-, both glycine and α-alanine; ⊗, both glycylglycine and glycylalanine.

Fig. 1.—Titration curves at 20°.

The results of Schmidt, Appleman and Kirk[23] on α-, β-, γ-, δ-amino substituted propionic and valeric acids show the same phenomenon. Thus for the series α-aminovaleric acid, β-aminopropionic acid, γ-aminovaleric acid and δ-aminovaleric acid, K^1_a = $10^{-2.4}$, $10^{-3.6}$, $10^{-4.0}$ and $10^{-4.2}$. The Derrick factors for the $-{}^{+}NH_3$ group in the α-, β-, γ- and δ-positions,

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[21] Since $H_3N^+CH_2COOH$ is a fairly strong acid, the use of the Derrick factor is to be preferred to a direct comparison of the logarithms of the dissociation constants.

[22] In Derrick's paper the influence of COOH appears to be persistent, but this is because the dissociation constants of symmetrical dibasic acids should be divided by two before they are compared with those of monobasic acids.

[23] Schmidt, Appleman and Kirk, *J. Biol. Chem.*, **81**, 723 (1929).

calculated from these results, are 1.0, 0.33, 0.2 and 0.14, respectively. The corresponding values for chlorine are 0.68, 0.19, 0.06, 0.02.[24]

This persistence of the influence of charged groups on dissociation constants is also shown by the fact that K^1_a and K^1_b of glycylglycine are noticeably greater than those of glycylglycylglycine. Another example of this generalization is that in the case of the dibasic saturated acids the first dissociation constants are almost exactly twice and the second are markedly less than one-half the dissociation constants of the corresponding fatty acids, when there are four or more atoms between the two carboxyl groups.

In their experiments the authors noted that alanine reacted like glycine and that glycylalanine like glycylglycine when these substances were treated with acid, but that this was not the case when base was added. Although the results of other investigators do not confirm this observation, it is shown so clearly in Fig. 1 and was equally apparent in the titration curves obtained at 0° that the authors believe that it is valid.

Summary

The acidic and basic dissociation constants of glycine, α-alanine, glycylglycine, and of glycylalanine were measured at two temperatures, 20 and 0°, by the electrometric method.

From these measurements the heats of acidic and basic dissociations of these amphoterics were calculated.

These values were used to calculate the dissociation constants at 25°.

The available data on the dissociation constants of these substance are collected together and where necessary the results have been extrapolated to 25°.

The authors note that the dissociation constants of these substances indicate that the influence of electrically charged groups on dissociation constants is not lessened to the same extent by intervening groups as is that of electrically neutral groups.

The authors' results indicate that an α-substituted methyl group influences the reaction of these substances with base to a much greater extent than their reaction with acid. This is not confirmed by the work of others in this field.

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[24] Derrick, THIS JOURNAL, **33,** 1167 (1911).