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The Thermodynamics of Ionization of Amino Acids. IV. The First Ionization Constants of Some Glycine Peptides[1]

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The thermodynamic functions for the ionization of the carboxyl groups of glycylglycine, glycyl-DL-alanine, glycyl-DL-α-amino-*n*-butyric acid, glycyl-L-leucine, glycyl-DL-serine and glycylasparagine were obtained at ten temperatures from measurements on cells without liquid junction. The large ionization constant and small decrease in entropy for the ionization of glycylglycine are consequences both of electrostatic effects and of strong interactions between the charged ammonium group and polar peptide linkage and water molecules. The peptides with an alkyl group on the α-carbon atom have more negative entropies and heats of ionization, an effect already observed in other series of acids. Solvation of the hydroxyl group in glycylserine and the amide group in glycylasparagine makes the entropies and heats of ionization of these acids larger than those of glycylglycine.

In two earlier papers of this series the ionization functions of N-acylamino acids[2] and N-carbamoylamino acids[3] were reported. These derivatives of α-amino acids can be represented by the formula $RCONHCH(R')COOH$ where R was CH_3 or C_2H_5 in the first series and NH_2 in the second and R′ was an alkyl group or hydrogen atom. These acids were stronger than the corresponding unsubstituted fatty acids. This was attributed to the interaction of the polar group and ionizing proton and to strong interactions of the ions and molecules of the acids with water. The entropy change associated with ionization was particularly sensitive to changes in solvation. Some interesting entropy and enthalpy effects were associated with substitution of alkyl groups for hydrogen on the α-carbon atom.

The present paper is a report of a similar study of N-glycyl-α-amino acids in which the terminal group (R) is $^+NH_3CH_2$. Because of the positive charge it bears this group can be expected to have a larger effect on the solvent than the terminal groups of the other series did. The other substituents (R′) in the present series include hydrogen, the alkyl groups CH_3, C_2H_5 and $CH_2CH(CH_3)_2$, and the polar, hydrogen-bonding CH_2OH and CH_2CONH_2 groups.

These glycine peptides are dipolar ions and the ionization of the carboxyl group converts a cation acid to the polar ion form

$$^+NH_3CH_2CONHCH(R')COOH + H_2O \rightleftharpoons {}^+NH_3CH_2CONHCH(R')COO^- + H_3O^+$$

The ionization of the ammonium group will be reported in a later paper.

The first ionization constants of the cationic form of these peptides (ZH^+) were obtained from measurements on cells of the type

$$(Pt)H_2\,|Z \doteq (m_1),\ ZH^+Cl^-(m_2)\,|AgCl\text{–}Ag \qquad (I)$$

Here m_1 and m_2 represent the stoichiometric molalities. Eighteen buffer solutions were used for the determination of the constants of glycylglycine, thirteen for glycylasparagine, and twelve for each of the others. Measurements were made at ten equally spaced temperatures between 5 and 50° in order to obtain accurate values of the heat and entropy of ionization and to locate the temperature at which the ionization constant reaches its maximum value.

Experimental

The apparatus has been described before.[2,4] With one exception the peptides were products of the H. M. Chemical Company, Santa Monica, California, and were used without further purification. Two samples of glycylserine were obtained from California Foundation for Biochemical Research, Los Angeles, California. One of these was used without purification. The other was dissolved in water, treated with Norite, and reprecipitated by addition of ethanol with noticeable improvement in color, crystallinity and purity. All of the peptides were subjected to the following tests: assay by formol titration with standard sodium hydroxide solution, water content by the Karl Fischer method, and various semi-quantitative tests.[5] The results are given in Table I. Though the glycylserine was not as pure as the other peptides the two samples of different purity gave the same results in the cells. It is probable that the impurities are inert and without effect on the ionization constant. Other chemicals were prepared and standardized as in earlier work.[2,6] Buffer solutions were prepared on the morning of the first day of measurements from weighed portions of peptide, hydrochloric acid and water. The technique of measurement of the electromotive forces has been described before.[2,4,7] The electromotive forces of cells containing glycylasparagine increased during the period of measurement. Hydrolysis of the terminal amide group[8] would consume hydrogen ions and cause such an increase. The change was very noticeable at 45 and 50°. In some of the measurements that extended over two days the total change between the initial and final readings at 25° was 0.8 to 1.3 mv. The rate of increase of electromotive force at various

TABLE I

Acid	Assay	H_2O, %	Cl, %	Other impurities[a]
Glycylglycine	99.9_3	0.05	<0.004	
Glycylalanine	100.0	.05	< .02	
Glycyl-α-amino-*n*-butyric acid	99.8_6	.09	< .008	0.004% NH_4
Glycylleucine	99.9_1	.18	< .004	0.005% Fe
Glycylserine, untreated	99.5	.21	< .004	
Glycylserine, repurified	99.0_8	.21	< .006	
Glycyl-D-asparagine	100.2	.00	< .1	[b]
Glycyl-L-asparagine	99.9_0	.13	< .1	

[a] Ammonia, phosphate, iron or heavy metals if 0.004% or larger. [b] Phosphate test was omitted for lack of material.

(1) This investigation was supported by a research grant, H-1651, from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service. A preliminary report was given at the American Chemical Society Meeting, Atlantic City, N. J., in September, 1956.

(2) E. J. King and G. W. King, THIS JOURNAL, **78**, 1089 (1956).

(3) E. J. King, *ibid.*, **78**, 6020 (1956).

(4) E. J. King, *ibid.*, **73**, 155 (1951).

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

(6) E. J. King, THIS JOURNAL, **76**, 1006 (1954).

(7) E. J. King and G. W. King, *ibid.*, **74**, 1212 (1952).

(8) S. J. Leach and H. Lindley, *Trans. Faraday Soc.*, **49**, 921 (1953).

temperatures was noted. Some of the cells were purposely taken over the lower part of the temperature range first, others over the higher. With all these data at hand it was possible to correct the electromotive forces back to the time of preparation of the solutions with a maximum uncertainty of 0.2 mv., the equivalent of about 0.0035 in pK. There is no evidence of hydrolysis of the peptide bond in any of these measurements. Initial and final readings at 25° usually agreed within 0.1 mv. except for cells containing glycylasparagine.

Though the original electromotive forces were used in the following calculations, it is necessary to give smoothed values here to save space. The electromotive forces can be represented as a function of temperature by

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

The parameters for this equation when electromotive forces are in absolute volts, concentrations are in moles per kilogram of water, and the hydrogen pressure is one atmosphere are given in Table II. The standard deviations between observed values and those calculated with equation 1 are ± 0.021 mv. for glycylglycine, ±0.042 for glycylalanine, ± 0.041 for glycylaminobutyric acid, ±0.036 for glycylleucine, ±0.053 for glycylserine, and ±0.055 for glycylasparagine.

TABLE II

PARAMETERS OF EQUATION 1

m_2	E_{25}	10^6a	-10^6b
	Glycylglycine		
	$m_1 = 0.5000m_2$		
0.01003	0.52166	368	100
.02003	.50234	302	75
.03012	.49168	267	55
.04016	.48456	245	62
.05009	.47912	226	49
.07009	.47108	200	50
	$m_1 = m_2$		
0.00999	0.53510	411	72
.01999	.51754	353	55
.03004	.50778	321	51
.05006	.49598	282	57
.07026	.48828	257	45
.09995	.48043	230	52
	$m_1 = 2.000m_2$		
0.01002	0.55107	467	74
.02008	.53453	411	55
.02999	.52535	378	45
.04011	.51886	358	41
.05016	.51395	340	42
.06928	.50690	316	43
	Glycyl-DL-alanine		
	$m_1 = m_2$		
0.005804	0.55042	533	81
.009588	.53680	495	96
.01893	.51969	440	90
.02722	.51092	413	95
.03809	.50305	388	85
.04643	.49852	374	90
	$m_1 = 2.000m_2$		
0.01013	0.55157	550	102
.01521	.54179	516	85
.02001	.53542	496	85
.02882	.52704	468	78
.03894	.52033	446	80
.04735	.51596	432	82
	Glycyl-DL-α-amino-n-butyric acid		
	$m_1 = 0.5000m_2$		
0.01001	0.52241	451	134
.02004	.50313	396	110
.03085	.49197	362	100
.04003	.48554	344	105
.05014	.48002	328	95
.07006	.47208	303	104
	$m_1 = m_2$		
0.01003	0.53586	508	110
.02002	.51842	454	91
.02991	.50883	422	75
.05036	.49679	387	78
.07041	.48926	364	87
.10005	.48146	340	92
	Glycyl-L-leucine		
	$m_1 = 0.5000m_2$		
0.01000	0.52372	464	125
.01502	.51224	430	108
.02001	.50456	407	100
.03000	.49426	377	92
.03986	.48719	356	92
.04907	.48219	342	90
	$m_1 = m_2$		
0.01000	0.53732	521	108
.01973	.52032	470	98
.02501	.51462	450	90
.03003	.51041	440	100
.03944	.50413	419	85
.04926	.49905	403	88

Glycyl-DL-serine

m_1	m_2	E_{25}	10^6a	-10^6b
0.004886	0.009771	0.51546	330	112
.01003	.02006	.49443	258	87
.01501	.03007	.48351	222	79
.02001	.04002	.47603	198	65
.02496	.04988	.47042	180	56
.03508	.07015	.46203	151	50
.01004	.01004	.52673	366	75
.02134	.01997	.51033	312	66
.03012	.03012	.49869	270	41
.05358	.05014	.48829	238	46
.06482	.06482	.48065	214	48
.10287	.09628	.47327	189	32
		Glycyl-D-asparagine		
0.005070	0.01002	0.51321	329	94
.03544	.07002	.45997	160	62
.005007	.005007	.54494	433	79
.01002	.01002	.52486	368	51
.01468	.01468	.51476	335	45
.02007	.02007	.50672	308	30
.02960	.02960	.49705	283	50
		Glycyl-L-asparagine		
0.009961	0.02004	0.49267	262	86
.02489	.05008	.46814	186	85
.04983	.10026	.45114	131	54
.01004	.01004	.52516	370	50
.01724	.01724	.51084	322	36
.03009	.03009	.49697	276	38

TABLE III

THE NEGATIVE LOGARITHMS OF THE FIRST IONIZATION CONSTANTS OF GLYCINE PEPTIDES AND THE PARAMETERS OF EQUATION 4

Temp., °C.	Glycyl-glycine	Glycyl-alanine	Glycyl-α-amino-n-butyric acid	Glycyl-leucine	Glycyl-serine	Glycyl asparagine
5	3.1574	3.1364	3.1325	3.1532	3.0066	2.9678
10	3.1495	3.1400	3.1358	3.1586	2.9965	2.9580
15	3.1444	3.1424	3.1407	3.1633	2.9889	2.9520
20	3.1409	3.1460	3.1457	3.1688	2.9831	2.9433
25	3.1397	3.1532	3.1546	3.1800	2.9808	2.9420
30	3.1410	3.1613	3.1635	3.1897	2.9789	2.9423
35	3.1420	3.1706	3.1729	3.1998	2.9787	2.9436
40	3.1459	3.1792	3.1838	3.2120	2.9805	2.9469
45	3.1519	3.1910	3.1973	3.2258	2.9863	2.9538
50	3.1599	3.2030	3.2115	3.2412	2.9924	2.9590
Std. dev.	0.00054	0.00071	0.00069	0.00084	0.00075	0.00264
A	1003.35	691.79	727.94	718.40	1090.92	1176.54
B	3.5670	1.8996	2.2214	2.1910	4.1993	4.8520
C	0.011207	0.0091655	0.0098400	0.0099294	0.011807	0.012907

TABLE IV

THERMODYNAMIC PROPERTIES ASSOCIATED WITH THE IONIZATION OF $^{+}NH_3CH_2CONHCH(R)COOH$

Acid	-R	$\Delta F^0_{298.16}$	$\Delta H^0_{298.16}$	$\Delta S^0_{298.16}$	$\Delta C^0_{298.16}$	θ°K.	pK_θ
Glycylglycine	-H	4283.3	+ 32	−14.26	−31	299	3.1395
Glycylalanine	$-CH_3$	4302.2	−563	−16.32	−25	275	3.1366
Glycyl-α-amino-n-butyric acid	CH_2CH_3	4302.8	−672	−16.69	−27	272	3.1313
Glycylleucine	$-CH_2CH(CH_3)_2$	4336.9	−752	−17.07	−27	269	3.1506
Glycylserine	$-CH_2OH$	4065.4	+189	−13.00	−32	304	2.9786
Glycylasparagine	$-CH_2CONH_2$	4014.3	+133	−13.01	−35	302	2.9418
Minimum probable errors		±2.1	±16	±0.06	±2		

Calculations and Results

The negative logarithm of the thermodynamic ionization constant, pK_1, is the limit at zero ionic strength of the function pK_1' defined by[9]

$$pK_1' = -\log [(m_1 + m_H')m_H'/(m_2 - m_H')] \quad (2)$$

The apparent hydrogen ion concentration is obtained from the data for Cell I by the relation[9]

$$-\log m_H' = (\mathfrak{F}/2.3026RT)(E_I - E^0_w) + \log m_2 - [2S\sqrt{\mu d_0}/(1 + A\mathring{a}\sqrt{\mu d_0})] \quad (3)$$

The working standard electromotive forces (E^0_w) are appropriate for the cells and techniques of this Laboratory.[2,3] The last term on the right-hand side of equation 3 is the Debye–Hückel approximation for the activity coefficient product of hydrochloric acid in the buffer solution. The ion size parameter $\mathring{a}$ was taken to be 4.00 A. This is an extrathermodynamic choice but a reasonable one. For pure hydrochloric acid the ion size parameter is 4.3 Å.[10] The activity coefficient product of hydrochloric acid is required for a mixture in which the concentration of HCl is m_H, that of ZHCl is $m_2 - m_H$, and that of $Z^{\pm}$ is $m_1 + m_H$. Suppose the effect of ZHCl on the activity coefficient to be similar to that of sodium chloride[11] and the effect of $Z^{\pm}$ to be similar to that of taurine.[12] The combined effect of these is equivalent to lowering $\mathring{a}$ from 4.3 to 4.0. Fortunately the effect of $\mathring{a}$ on the value of pK is relatively small for acids with pK values around three.[13] For example, a shift of $\mathring{a}$ from 4.00 to 5.00 increases pK_1 of glycylalanine by only 0.0013.

The values of pK_1' at each buffer ratio, m_1/m_2, were extrapolated to zero ionic strength. The intercepts for the different buffer ratios were then averaged and are recorded in Table III. The standard derivations listed in the table are the errors in each set of intercepts as obtained by the method of least squares. They indicate the precision of the extrapolations. For glycylasparagine the pK_1' values for the L-isomer were on the average about 0.005 unit higher than those for the D-compound at all temperatures. Since this is not far outside the combined uncertainties, it cannot be asserted definitely that this represents a real difference in behavior of the two isomers. For that reason a separate extrapolation for each isomer was not attempted.

The parameters of the Harned and Robinson equation[14] are also included in Table III. The

$$pK_1 = (A/T) - B + CT \quad (4)$$

thermodynamic functions calculated with these parameters[9,14] are given in Table IV. They include the minimum pK value (pK_θ) at absolute temperature θ and the change in free energy (ΔF_1^0), enthalpy (ΔH_1^0), entropy (ΔS_1^0), and heat capacity (ΔC_{p1}^0) associated with the ionization reactions in the standard state. Throughout this paper the

(9) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 2nd ed., Reinhold Publ. Corp., New York, N. Y., 1950, pp. 505–506.

(10) Ref. 9, pp. 380–381.

(11) Ref. 9, pp. 450–454.

(12) E. J. King, THIS JOURNAL, **75**, 2204 (1953).

(13) R. G. Bates, *J. Research Natl. Bur. Standards*, **47**, 127 (1951); ref. 7.

(14) H. S. Harned and R. A. Robinson, *Trans. Faraday Soc.*, **36**, 973 (1940).

free energy and enthalpy changes are given in defined calories per mole and the entropy and heat capacity changes in calories per degree-mole. The minimum probable errors at the bottom of Table IV correspond to random errors in the electromotive force measurements and preparation of the solutions.[2] Because of the low purity of glycylserine the uncertainties in its thermodynamic functions are necessarily larger, though probably not more than three times as large. For glycylasparagine uncertainty results not only from the change in electromotive force with time but also from the apparent difference in behavior of the two isomers. If pK_1 of the L-isomer is consistently higher by 0.005 this would make ΔF_1^0 higher by 7 cal./mole and ΔS_1^0 more negative by 0.02 cal./deg.-mole at 25°.

There are few accurate values of the thermodynamic functions with which the present ones can be compared, though there are all too many approximate values obtained by potentiometric titrations. Neuberger[15] used cells with liquid junction and a double extrapolation to obtain $pK_1 = 3.083$ at 25° for glycylglycine. Smith and Smith[16] used the same method as the present investigation. They used only seven buffer solutions, did not exclude oxygen, and measured the electromotive forces only to the nearest 0.1 mv. They obtained for pK_1 the value 3.151, for ΔH^0, 394, and for ΔS^0, −13.10 at 25°. Kilpi[17] has recently obtained the value 3.11 for pK_1 of glycylglycine by extrapolation of measurements in potassium chloride solutions. His value of the enthalpy change, 1600 cal./mole, is in poor agreement with the above values.

Discussion

Comparison of Glycylglycine with Other Acids.— From one point of view glycylglycine can be considered as a derivative of acetic acid, RCH_2COOH, where R− is the strongly polar, hydrogen-bonding group $^+NH_3CH_2CONH-$. The thermodynamic functions of a number of such acids are given in Table V.

TABLE V

THERMODYNAMIC FUNCTIONS FOR THE IONIZATION OF RCH_2COOH AT 25°

Acid	−R	pK	ΔH^0	ΔS^0	Ref.
Hydrogen malonate ion	$-COO^-$	5.699	−1160	−30.0	18
Acetic acid	−H	4.757	− 100	−22.1	19
Iodoacetic acid	−I	3.174	−1370	−19.1	20
N-Acetylglycine	$-NHCOCH_3$	3.670	− 150	−17.3	2
Bromoacetic acid	−Br	2.901	−1200	−17.2	20
Glycolic acid	−OH	3.832	+ 180	−16.9	21
Hydantoic acid	$-NHCONH_2$	3.876	+ 290	−16.8	3
Chloroacetic acid	−Cl	2.867	−1080	−16.8	20
Fluoroacetic acid	−F	2.585	−1350	−16.3	20
Glycylglycine	$-NHCOCH_2NH_3^+$	3.140	+ 30	−14.3	
Cyanoacetic acid	−CN	2.468	− 840	−14.1	22
Glycine	$-NH_3^+$	2.349	+ 950	− 7.6	4

(15) A. Neuberger, *Proc. Roy. Soc. (London)*. **A158**, 68 (1937).

(16) E. R. B. Smith and P. K. Smith, *J. Biol. Chem.*, **146**, 187 (1942); their results have been recalculated and fitted to the Harned and Robinson equation.[14]

(17) S. Kilpi, *Suomen Kemistilehti*, **29B**, 113 (1956); *Referat. Zhur. Khim.*, 3909 (1957).

(18) W. J. Hamer, J. O. Burton and S. F. Acree, *J. Research Natl. Bur. Standards*, **24**, 269 (1940).

(19) H. S. Harned and R. W. Ehlers, THIS JOURNAL, **55**, 652 (1933).

The grosser variations of pK can be interpreted as originating in the electrostatic interaction of the charged or polar substituent with the proton of the carboxyl group partly through the solvent and partly through the cavity of the molecule itself.[24] Yet it is difficult to explain some of the finer features of acid behavior by classical electrostatic interaction.[20] Cyanoacetic acid, for example, is stronger than the halogenoacetic acids because of its abnormally small entropy decrease.[22] The comparatively low pK value of glycylglycine nevertheless can be interpreted qualitatively as a consequence of the presence of a polar peptide linkage close to the carboxyl group and a charged ammonium group further away. Glycylglycine is therefore a stronger acid than acetylglycine which contains the peptide linkage but not the ammonium group. It is a weaker acid than glycine in which the positive charge is closer to the carboxyl group.

The slightly positive heat of ionization of glycylglycine is characteristic of acids containing a positively charged substituent (glycine) or of those in which the terminal group is hydrogen-bonding (glycolic and hydantoic acids).

The entropy of ionization of glycylglycine is less negative than those of acetylglycine and hydantoic acid but more negative than that of glycine. The decrease in entropy associated with ionization of carboxyl groups is caused largely by orientation of water about the charges.[2] The proximity of the charged ammonium group and polar peptide group near the carboxylate ion reduces this effect and leads to a smaller decrease in entropy for glycylglycine than for acetic acid. The terminal charge is more effective in orienting water than the methyl group in acetylglycine and the amino group in hydantoic acid are, so that glycylglycine has a less negative entropy of ionization than the other two acids do.

The thermodynamic properties of glycylglycine are listed in Table VI in comparison with those of other acids having a five atom chain attached to the carboxyl group. The heat and entropy of ionization of *n*-hexoic acid are more negative than those of acetic acid (Table V). This has been attributed to a decrease in flexibility of the long hydrocarbon chain after ionization.[2,25,27] The structure-strengthening effect of alkyl groups on water also probably contributes to the decrease in entropy.[2] The introduction of a charged ammonium group at the end of the chain in δ-aminovaleric acid increases ΔH^0 and ΔS^0 and decreases pK. This will be caused in part by the electrostatic effect of the positive charge on the ionizing proton and in part by the structure-breaking effect of the ammonium group on water. The effective range of

(20) D. J. G. Ives and J. H. Pryor, *J. Chem. Soc.*, 2104 (1955). Values corrected to molal scale.[23]

(21) L. F. Nims, THIS JOURNAL, **58**, 987 (1936).

(22) F. S. Feates and D. J. G. Ives, *J. Chem. Soc.*, 2798 (1956). Values corrected to molal scale.[23]

(23) J. H. Asbby, E. M. Crook and S. P. Datta, *Biochem. J.*, **56**, 190 (1954).

(24) J. Kirkwood and F. Westheimer, *J. Chem. Phys.*, **6**, 506, 513 (1938); F. Westheimer and M. Shookhoff, THIS JOURNAL, **61**, 555 (1939); F. Westheimer and J. Kirkwood, *Trans. Faraday Soc.*, **43**, 77 (1947).

TABLE VI

THE THERMODYNAMIC PROPERTIES OF SOME LONG CHAIN ACIDS AT 25°

Acid	Formula	pK	ΔH^0	ΔS^0
n-Hexoic acid[25]	$CH_3CH_2CH_2CH_2CH_2COOH$	4.856	−700	−24.6
δ-Aminovaleric acid[26]	$^+NH_3CH_2CH_2CH_2CH_2COOH$	(4.229)	(+160)	(−18.8)
N-Propionylglycine[2]	$CH_3CH_2CO\ NHCH_2COOH$	3.717	−140	−17.5
Glycylglycine	$^+NH_3CH_2CO\ NHCH_2COOH$	3.140	+ 30	−14.3

influence of the ammonium group is about 5 A.[28] Thus in spite of the location of the ammonium and carboxylate ions at opposite ends of the chain, their regions of influence on the solvent will overlap. The region over which the carboxylate ion alone holds sway is thereby reduced and the entropy change on ionization is less negative than that of *n*-hexoic acid. With N-propionylglycine the closeness of the peptide group to the carboxyl group still further reduces the influence of the latter and the entropy decrease is still smaller. Finally, with glycylglycine the combined effect of terminal charge and peptide group leads to the least negative ΔS^0 value.

The peptide linkage may also act as a chain stiffener[2] if it is largely restricted to the *trans* configuration. The effect of ionization on the flexibility of the chain would thus be much smaller for propionylglycine and glycylglycine than for hexoic acid, and these acids would have less negative entropies of ionization. Edsall, Otvos and Rich[29] have noted that some of the stretching frequencies of the chains in ω-amino acids are affected by ionization. They also report a Raman line at 922 cm.$^{-1}$ for glycylglycine which is not found for the cationic form. They suggest that the dipolar ion form may have additional configurations of the chain not exhibited by the cation. These may result from rotations of the terminal NH_3^+ and COO^- groups around the bonds to the neighboring carbon atoms or from formation of a *cis* ring structure. Ellenbogen[30] has estimated the potential barriers to such rotation for the alanylalanines and concludes that the *trans* configuration is more stable. The entropies of ionization of the alanylalanines are about the same as that of glycylalanine. Ellenbogen considers that these values indicated that ionization is accompanied by folding of the chain. This hypothesis is based on the assumption that the normal entropy of ionization of a carboxyl group is −7 cal.deg.$^{-1}$mole^{-1} as for glycine and does not take into consideration the sensitivity of ΔS^0 to the groups in the immediate vicinity of the carboxyl group. Folding or *cis* ring structures should certainly be more probable for glycylglycine with its terminal positive charge than for propionylglycine. Yet the latter has the more negative entropy of ionization. Though folding and formation of *cis* ring structures may possibly occur, it is unlikely that they make important contributions to ΔS^0.

Chain Branching Effects.—Earlier papers[2,3] have called attention to characteristic decreases in entropy and enthalpy associated with the introduction of an alkyl group for a hydrogen atom on the α-carbon atom. The present series of acids includes the α-methyl, -ethyl and -isobutyl derivatives of glycylglycine. As the data in Table IV show, ΔS^0 and ΔH^0 become more negative as the alkyl group gets bulkier. The changes in thermodynamic properties caused by introduction first of a methyl for hydrogen and then of an ethyl for methyl are summarized in Tables VII and VIII. Though the changes in entropy and enthalpy are well defined, they are so closely coupled that the variations in pK are remarkably small.

TABLE VII

SUBSTITUTION OF METHYL FOR HYDROGEN ON THE α-CARBON ATOM AT 25°

A = $XCH(CH_3)COOH$ B = XCH_2COOH

Series	X	$\Delta S_A^0 - \Delta S_B^0$	$\Delta H_A^0 - \Delta H_B^0$	$pK_A - pK_B$
Fatty acids[25]	CH_3	−1.79	−570	−0.027
N-Acetylamino acids[2]	$NHCOCH_3$	−1.83	−480	+ .045
N-Carbamoylamino acids[2]	$NHCONH_2$	−1.83	−620	.016
N-Glycylamino acids	$NHCOCH_2NH_3^+$	−2.06	−600	.013
α-Amino acids[31]	NH_3^+	−0.99	−300	− .008
α-Hydroxyl acids[32]	OH	−1.06	−270	+ .030

TABLE VIII

SUBSTITUTION OF ETHYL FOR METHYL ON THE α-CARBON ATOM AT 25°

A = $XCH(C_2H_5)COOH$ B = $XCH(CH_3)COOH$

Series	X	$\Delta S_A^0 - \Delta S_B^0$	$\Delta H_A^0 - \Delta H_B^0$	$pK_A - pK_B$
N-Acetylamino acids[2]	$NHCOCH_3$	−0.47	−140	+0.001
N-Carbamoylamino acids[2]	$NHCONH_2$	−0.84	−260	− .006
N-Glycylamino acids	$NHCOCH_2NH_3^+$	−0.37	−110	+ .001
α-Amino acids[31]	NH_3^+	−1.12	−400	− .050

The origin of these effects has been sought in the interactions between alkyl groups and water molecules.[2] About each carboxylate ion there are probably two kinds of hydration sheaths. The innermost one is a monomolecular layer of water hydrogen bonded to the oxygen atoms of the carboxylate ion. Water in this region has an abnormally low entropy. Outside of this is a region of disordered, high entropy water[33,34] which eventually meshes

(25) D. Everett, D. Landsman and B. Pinsent, *Proc. Roy. Soc. (London)*, **215A**, 403 (1952).

(26) These values were estimated from those of the other ω-amino acids using the linear variation in each property with the reciprocal of the number of carbon atoms between the ammonium and carboxyl groups.[3]

(27) A. Evans and S. Hamann, *Trans. Faraday Soc.*, **47**, 34 (1951).

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(29) J. T. Edsall, J. W. Otvos and A. Rich, THIS JOURNAL, **72**, 474 (1950).

(30) E. Ellenbogen, *ibid.*, **78**, 369 (1956).

(31) Glycine, ref. 4; alanine, L. F. Nims and P. K. Smith, *J. Biol. Chem.*, **101**, 401 (1933); P. K. Smith, A. C. Taylor and E. R. B. Smith, *ibid.*, **122**, 109 (1937).

(32) Glycolic acid, ref. 21; lactic acid, L. F. Nims and P. K. Smith, *ibid.*, **113**, 145 (1936).

(33) H. S. Frank and M. Evans, *J. Chem. Phys.*, **13**, 507 (1945).

(34) Feates and Ives[22] have supposed that the inner sheath of low entropy water is several molecular layers thick. The microwave measurements of Harris and O'Konski (*J. Phys. Chem.*, **61**, 310 (1957)) do not support this.

into the normal water structure at a distance from the ion. Alkyl groups on the α-carbon atom penetrate this outer layer and by their structure-strengthening action lower its entropy.[2] This makes the entropies of alkyl-substituted anions more negative than that of the unsubstituted anion. With glycine the proximity of the two charges may give rise to a thicker inner low-entropy layer.[3] If a methyl group penetrates this only partially its structure-strengthening effect will be reduced while that of the longer ethyl group will be increased. This would explain the anomalous fact that substitution of ethyl for methyl produces a larger effect than that of methyl for hydrogen in the amino acid series. It does not account for the small change between glycolic and lactic acids.

Decreases in entropy of the alkyl-substituted anions may also be caused by restriction in rotation of the alkyl groups in the rigid sheaths of water in which they become embedded after ionization.[2]

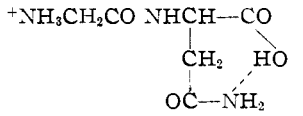
The Effect of Polar Substituents.—The effect of substituting a CH_2OH for a CH_3 group is shown in Table IX. There is somewhat less regularity in behavior than with alkyl substitution, yet the different pairs show roughly comparable effects. The increases in ΔS^0 and ΔH^0 are probably caused by the ability of hydroxyl groups to tie down water even before ionization and thereby reduce the entropy decrease associated with formation of the carboxylate ion.

TABLE IX

SUBSTITUTION OF CH_2OH FOR CH_3 ON THE α-CARBON ATOM AT 25°

A	B	$\Delta S_A^0 - \Delta S_B^0$	$\Delta H_A^0 - \Delta H_B^0$	$pK_A - pK_B$
Glycylserine	Glycyl-α-alanine	3.3	750	−0.173
Serine[35]	α-Alanine	2.8	620	− .162
Threonine[35]	α-Amino-*n*-butyric acid[31]	3.8	840	− .200
Allothreonine[35]	α-Amino-*n*-butyric acid	2.4	450	− .192

The close agreement between the entropies of ionization of glycylserine and glycylasparagine is noteworthy, though in view of the probable error in each value the almost exact agreement cannot be taken too seriously. Leach and Lindley[8] found that the hydrolysis of glycylasparagine is first order in the cation acid alone and that it has a low entropy of activation. They suggested a unimolecular mechanism in which the cationic form has an internally hydrogen-bonded structure not greatly different from that of the activated complex



The dipolar form in crystals has a very different structure with the glycylamino group almost perpendicular to the planar succinamide part of the molecule.[36] The terminal amino group of the amide and the carboxyl group are thus far apart in the crystal. It is possible that ionization might involve considerable internal rotations in passing from the hydrogen-bonded structure shown above to that of the dipolar ion. This would lead to a smaller entropy decrease for glycylasparagine than for the other peptides. Such an explanation for the similar entropy change of glycylserine is not as probable. It seems simpler to attribute the small entropy decrease of these two acids to solvation of the polar groups.

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