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Physico-Chemical Studies of the Simpler Polypeptides. I. The Dissociation Constants of Mono-, Di-, Tri-, Tetra-, Penta-, Hexa- and Hepta-glycine and their Esters[1]

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The results of Pacsu[1a] on the condensation of peptide esters, together with the observations of others,[2] suggest the possibility of discontinuities in the structures of the simple polypeptides. Some support for this view is provided by X-ray measurements,[3] by solubility data,[4] and by studies on the dielectric increment of the polyglycines.[5] Information concerning the distance apart of the polar ends of an amino-acid can be obtained from the dissociation constants of the end-groups, and this subject has been considered with reference to glycine and diglycine by Neuberger[6] and by Westheimer and Shookhoff.[7] The object of the present work was to see whether any indication of structural changes could be obtained from the dissociation constants of the first seven members of the glycine series. Accurate determinations have been made with glycine and diglycine[8] and approximate measurements of tri-, tetra-, penta- and hexa-glycine have been reported by Stiasny and Scotti.[9] The latter titrated 0.05 *N* solutions of tetra- and penta-glycine with *N* hydrochloric acid and *N* sodium hydroxide, and in the case of the hexa-acid they worked with suspensions; the results for these substances cannot, therefore, be regarded as satisfactory. Determinations of the dissociation constants of the first seven glycines have therefore been made, the glass electrode being employed in order to avoid possible difficulties with the hydrogen electrode, due to adsorption or reduction, especially when working with the higher peptides. Measurements also have been made with the methyl or ethyl esters of the amino-acids, with the exception of those of penta- and hepta-glycine which could not be prepared, for reasons which will be examined in another paper.

Experimental

Materials.—A good commercial specimen of glycine was converted into the ethyl ester hydrochloride,[10] then into dioxopiperazine[11] and finally into diglycine.[12] From this, triglycine was obtained by the reaction first with chloroacetyl chloride and then with ammonia.[13] Tetraglycine was prepared from the Curtius "biuret base,"[2d] resulting when a solution of pure glycine ethyl ester[14] in dry ether was allowed to stand for some weeks, in the following manner. Sufficient concentrated hydrochloric acid was added to a suspension of the material in water to dissolve the tetraglycine ethyl ester, and the dioxopiperazine remaining was filtered off. To the filtrate was added 30% sodium hydroxide solution to make the solution slightly alkaline and the whole was heated to 60° for forty-five minutes. Hydrochloric acid was then added until the mixture was just acid to methyl red, and the tetraglycine was precipitated by alcohol. Tetraglycine was converted into pentaglycine by the action of chloroacetyl chloride and ammonia, in two stages.[15] The hexaglycine employed in this work was obtained by the hydrolysis of a specimen of hexaglycine methyl ester, for the gift of which we wish to thank Professor E. Pacsu; this was prepared from triglycine methyl ester[2b] by condensation in methyl alcohol solution. Finally, hexaglycine was converted into heptaglycine by means of chloroacetyl chloride and ammonia, in the usual manner.

With the exception of hexaglycine methyl ester, the hydrochlorides of the esters were obtained from the corresponding acids by the action of hydrogen chloride in ethyl alcohol.[10,16] The hydrochloride of hexaglycine methyl ester was prepared by dissolving the pure ester, referred to above, in an equivalent amount of hydrochloric acid. Attempts to prepare pentaglycine ester proved unsuccessful. Mono-, di- and tri-glycine were recrystallized from aqueous alcohol, and the other acids were purified by Fischer's method,[2b] involving evaporation with dilute ammonia. The ester hydrochlorides of mono-, di-, tri- and tetra-glycine were recrystallized from alcoholic hydrogen chloride, care being taken to avoid prolonged heating. The purity of all the substances was checked by means of formol titrations carried out potentiometrically with the glass electrode. In spite of repeated crystallizations and other attempts at purification, it was not found

(1) This investigation was carried out with the support of a grant from the Penrose Fund of the American Philosophical Society to Professor E. Pacsu.

(1a) Pacsu, *Nature*, **144**, 551 (1939); and unpublished experiments.

(2) Fischer, (a) *Ber.*, **37**, 2501 (1901); (b) **39**, 453 (1906); Curtius, (c) *ibid.*, **16**, 734 (1883); (d) **37**, 1283 (1904); (e) *J. prakt. Chem.*, **37**, 173 (1888).

(3) *Cf.*, Meyer and Go, *Helv. Chim. Acta*, **17**, 1488 (1934).

(4) Cohn, McMeekin, Edsall and Weare, THIS JOURNAL, **56**, 2270 (1934); McMeekin, Cohn and Weare, *ibid.*, **57**, 626 (1935); **58**, 2173 (1936).

(5) (a) Wyman and McMeekin, *ibid.*, **55**, 908 (1933); (b) Wyman, *ibid.*, **56**, 536 (1934); (c) see also Wyman, *Chem. Rev.*, **19**, 213 (1936).

(6) Neuberger, *Proc. Roy. Soc.*, **158A**, 68 (1937).

(7) Westheimer and Shookhoff, THIS JOURNAL, **61**, 555 (1939).

(8) (a) Harned and Owen, *ibid.*, **52**, 5091 (1930); (b) Owen, *ibid.*, **56**, 24 (1934).

(9) Stiasny and Scotti, *Ber.*, **63**, 2977 (1930).

(10) Harries and Weiss, *Ann.*, **327**, 365 (1903).

(11) Fischer, *Ber.*, **39**, 2893 (1906).

(12) Fischer, *ibid.*, **38**, 605 (1905).

(13) Fischer, *ibid.*, **36**, 2982 (1903); **37**, 2500 (1904).

(14) Fischer, *ibid.*, **34**, 433 (1901).

(15) Fischer, *ibid.*, **37**, 2486 (1904).

(16) Fischer, *ibid.*, **34**, 2868 (1901); **36**, 2984 (1903).

possible to obtain pentaglycine in a pure state; the dissociation constants, however, were found to be independent of the amount of impurity, and hence it seemed probable that the latter was a neutral substance whose presence would have little effect on the results.

Measurement of pH.—The *p*H's of partially neutralized solutions of the various amino-acids and esters were determined with a Leeds and Northrup glass electrode outfit. The electrode was standardized by means of Clark and Lubs buffer solutions, phthalate solutions being used for the *p*H range of 3 to 4, and borate solutions from *p*H 7.8 to 9.6. It is possible that the recorded values for the *p*H's of these buffers may be about 0.03 unit too low; if so, the *pK* values given in this paper will have to be increased by a similar amount. The test solutions were contained in a glass beaker waxed internally, standing on wax blocks, to avoid errors due to electrical leakage. Stirring was carried out by means of a stream of nitrogen led in through a waxed tube. The initial concentrations of mono-, di- and tri-glycine were 0.1 *N*, while those of tetra- and penta-glycine were about 0.05 and 0.01 *N*, respectively. The sodium hydroxide and hydrochloric acid used in the titrations had the same concentration as the solutions being titrated. Owing to the sparing solubility of hexa- and hepta-glycine, and the liability of errors arising from the use of very dilute solutions, special methods were adopted in the study of these substances. The hexa-glycine was dissolved in either sodium hydroxide or hydrochloric acid, so as to make a 0.01 *N* solution, and this was then back-titrated. Since the hydrochloride of hepta-glycine was also sparingly soluble, it was necessary to dissolve the amino-acid in 0.01 *N* sodium hydroxide for both titrations. In one case the partial back titration by hydrochloric acid was carried out in the normal manner, while in the other excess of the acid was added rapidly, so as to give a mixture of equivalent amounts of the amino-acid and its hydrochloride, and the *p*H determined immediately. Measurements were thus always made with homogeneous solutions, although some of those involving hepta-glycine may have been supersaturated.

The initial concentrations of the ester hydrochlorides were all 0.05 *N*, with the exception of the hexa-glycine ester which was slightly more dilute; 0.05 *N* sodium hydroxide was employed in the titration.

The data recorded in this paper are all for a temperature of 20°.

Calculations.—Provided the concentration of OH^- can be neglected in comparison with that of H^+, as is the case when a free amino-acid is neutralized by a strong acid, it is possible to write[17]

$$pK_1 = pH - \log\left(\frac{C}{A - c_{H^+}} - 1\right) - \log\frac{\gamma_{R^\pm}}{\gamma_{R^+}} \quad (1)$$

where K_1 is, by convention, the dissociation constant of the carboxylic acid $^+NH_3RCOOH$, and pK_1 is $-\log K_1$. The *p*H is here defined as $-\log a_{H^+}$, where a_{H^+} is the activity and c_{H^+} is the concentration of hydrogen ions in a solution consisting of C g. equiv. of amino-acid to which A g. equiv. of strong acid has been added. The activity coefficients $\gamma_{R^\pm}$ and γ_{R^+} refer to the dipolar ion, $^+NH_3RCOO^-$, and to $^+NH_3RCOOH$, respectively, since the undissociated acid is almost exclusively in the dipolar ion form. Similarly, for the neutralization of the free amino-acid by a strong base

$$pK_2 = pH + \log\left(\frac{C}{B - c_{OH^-}} - 1\right) - \log\frac{\gamma_{R^-}}{\gamma_{R^\pm}} \quad (2)$$

where pK_2 is now the acidic dissociation constant of the ammonium ion acid $^+NH_3RCOO^-$. The terms *p*H, C and $\gamma_{R^\pm}$ have the same significance as in equation (1), and in addition B is the concentration of added strong base, c_{OH^-} is the concentration of hydroxyl ions, and γ_{R^-} is the activity coefficient of the NH_2RCOO^- ions. In dilute solutions $-\log\gamma_{R^\pm}$ is proportional to the ionic strength,[18] but $-\log\gamma_{R^+}$ and $-\log\gamma_{R^-}$ are, according to the Debye–Hückel theory, linear functions of the square root of the ionic strength; the activity coefficient ratios $\gamma_{R^\pm}/\gamma_{R^+}$ and $\gamma_{R^-}/\gamma_{R^\pm}$ will therefore, in general, differ from unity. In view of the approximations which have to be made, *e. g.*, the derivation of $-\log a_{H^+}$ from the measured potential of the glass electrode on the assumption that liquid junction potentials are completely eliminated, the values of $\log\gamma_{R^\pm}/\gamma_{R^+}$ and $\log\gamma_{R^-}/\gamma_{R^\pm}$ will be taken as zero; the error introduced by this approximation is small, since in none of the solutions used in the present work did the ionic strength exceed 0.1. In order to calculate c_{H^+} from a_{H^+}, obtained in the manner just indicated, the activity coefficient of H^+ was assumed to be equal to the mean activity coefficient of hydrochloric acid at the same ionic strength. For the evaluation of c_{OH^-}, the ionic activity product of water was taken as 0.68×10^{-14} at 20°,[19] and the activity coefficient of OH^- was assumed to be equal to the mean value for sodium hydroxide. The solutions were generally so dilute, however, that no serious error would have been made if c_{H^+} and c_{OH^-} had been taken as equal to a_{H^+} and a_{OH^-}, respectively. A selection of the results obtained in the manner described is recorded in Tables I, II and III.

Results

When the free amino-acid exists almost exclusively in the dipolar ion form, as is the case with

(17) See Hitchcock, "The Chemistry of the Amino Acids and Proteins," C. C. Thomas, Springfield, Ill., edited by Schmidt, 1938, p. 605.

(18) Kirkwood, *J. Chem. Phys.*, **2**, 351 (1934); *Chem. Rev.*, **24**, 233 (1939).

(19) Harned and Hamer, THIS JOURNAL, **55**, 2194 (1933).

TABLE I

POTENTIOMETRIC TITRATIONS OF AMINO-ACIDS WITH HYDROCHLORIC ACID

C (g. equiv./liter)	A	pH	$-\log c_{H^+}$	pK_1
		Glycine		
0.0769	0.0231	2.76	2.70	2.34
.0667	.0333	2.45	2.38	2.33
.0588	.0412	2.21	2.13	2.34
.0555	.0445	2.10	2.02	2.33
		Diglycine		
.0800	.0200	3.69	3.63	3.21
.0667	.0333	3.19	3.12	3.17
.0572	.0428	2.80	2.72	3.20
		Triglycine		
.0833	.0167	3.80	3.75	3.19
.0667	.0333	3.20	3.13	3.18
.0667	.0333	3.23	3.16	3.21
		Tetraglycine		
.0385	.0115	3.52	3.47	3.13
.0333	.0167	3.13	3.08	3.09
.0294	.0206	2.84	2.78	3.10
.0263	.0237	2.56	2.50	3.11
		Pentaglycine		
.00780	.00156	3.73	3.71	3.06
.00675	.00271	3.42	3.40	3.14
.00632	.00316	3.29	3.27	3.14
.00505	.00454	2.87	2.84	3.07
		Hexaglycine		
.00690	.00414	3.13	3.09	3.10
.00645	.00322	3.32	3.28	3.18
.00572	.00171	3.58	3.54	3.10
.00361	.00174	3.39	3.36	3.15
		Heptaglycine		
.00357	.00178	3.23	3.20	2.92
.00336	.00167	3.27	3.24	2.96

TABLE II

POTENTIOMETRIC TITRATIONS OF AMINO-ACIDS WITH SODIUM HYDROXIDE

C (g. equiv./liter)	B	pH	$-\log c_{OH^-}$	pK_2
		Glycine		
0.0769	0.0231	9.62	4.68	9.79
.0714	.0286	9.63	4.46	9.81
.0667	.0333	9.78	4.31	9.78
.0625	.0375	9.98	4.10	9.81
		Diglycine		
.0800	.0200	7.77	6.33	8.25
.0667	.0333	8.21	5.88	8.21
.0572	.0421	8.70	5.38	8.23
		Triglycine		
.0833	.0167	7.51	6.60	8.11
.0714	.0286	7.96	6.13	8.14
.0667	.0333	8.09	6.00	8.09
.0555	.0445	8.72	5.36	8.11
		Tetraglycine		
.0333	.0165	8.06	6.11	8.07
.0294	.0204	8.42	5.75	8.06
.0263	.0235	9.06	5.11	8.04
		Pentaglycine		
.00632	.00316	7.99	6.18	7.99
.00594	.00357	8.23	5.94	8.05
.00562	.00393	8.43	5.74	8.06
.00505	.00454	8.94	5.23	7.98
		Hexaglycine		
.00769	.00500	7.96	6.21	7.69
.00714	.00393	7.78	6.39	7.69
.00625	.00218	7.46	6.71	7.73
.00271	.00165	7.84	6.33	7.65
.00257	.00106	7.46	6.71	7.62
		Heptaglycine		
.00667	.00333	7.65	6.52	7.65
.00667	.00333	7.74	6.43	7.74

TABLE III

POTENTIOMETRIC TITRATION OF ESTER HYDROCHLORIDES

Ester HCl. g. equiv./liter	NaOH	pH	pK_E
	Glycine ethyl ester		
0.0400	0.0100	7.36	7.84
.0333	.0167	7.82	7.82
.0285	.0214	8.27	7.79
.0270	.0229	8.61	7.86
	Diglycine ethyl ester		
.0385	.0115	7.57	7.94
.0333	.0165	7.90	7.91
.0294	.0205	8.27	7.91
	Triglycine ethyl ester		
.0385	.0115	7.55	7.92
.0333	.0165	7.90	7.91
.0294	.0205	8.27	7.91
	Tetraglycine ethyl ester		
.0385	.0115	7.65	7.83
.0333	.0167	7.80	7.80
.0312	.0187	7.97	7.79
	Hexaglycine methyl ester		
.0312	.0133	7.68	7.81
.0294	.0154	7.89	7.85
.0263	.0191	8.32	7.90
.0250	.0206	8.57	7.89

the glycines under consideration, the constant K_1, now called K_A by convention, refers to the dissociation of the carboxylic acid, *viz.*

$$^+NH_3RCOOH + H_2O \rightleftharpoons H_3O^+ + {}^+NH_3RCOO^-$$

while K_2, for which the symbol K_C is used, refers to the dissociation constant of the ammonium ion acid, *viz.*

$$^+NH_3RCOO^- + H_2O \rightleftharpoons H_3O^+ + NH_2RCOO^-$$

The quantity K_w/K_2 gives the basic dissociation constant of the conjugate amine base, NH_2RCOO^-.

The dissociation constant of the ammonium ion acid $^+NH_3RCOOH$ is called K_B; this quantity cannot be determined directly, but it is generally assumed to be the same as that for the acid $^+NH_3RCOOMe$ (or Et), *i. e.*, the ammonium ion acid corresponding to the methyl or ethyl ester of the amino-acid.[20] This assumption is justified by the fact that the basic dissociation constant of *p*-aminobenzoic acid is almost the same as that of its methyl and ethyl esters. The value of K_B is thus taken as equal to K_E, *i. e.*, the dissociation constant of the ammonium ion acid of the corresponding peptide ester, as given in Table III. Finally, the carboxylic acid dissociation constant of NH_2RCOOH, called K_D, is obtained from its equivalent quantity K_1K_2/K_B. The four possible acidic dissociation constants of the amino-acids can thus be derived from the data in Tables I, II and III. In addition, the constant for the equilibrium between uncharged molecules and dipolar ions, *viz.*

$$NH_2RCOOH \rightleftharpoons {}^+NH_3RCOO^-$$

readily can be obtained; it is called K_Z and is given by K_A/K_B or by K_D/K_C, under the conditions that the normal equilibrium lies well over to the right. The mean results for the seven amino-acids studied in this work are summarized in Table IV; the pK values are primed, since activity effects have not been completely eliminated.

TABLE IV

DISSOCIATION CONSTANT EXPONENTS AT 20°

	pK'_A	pK'_B	pK'_C	pK'_D	pK'_Z
Glycine	2.33[a]	7.83[m]	9.80[b]	4.30	5.50
Diglycine	3.19[c]	7.92[n]	8.23[d]	3.50	4.73
Triglycine	3.19[e]	7.91	8.11[f]	3.39	4.72
Tetraglycine	3.11[g]	7.81	8.06[h]	3.36	4.70
Pentaglycine	3.10[i]	..	8.02[j]	..	..
Hexaglycine	3.13[k]	7.86	7.69[l]	2.96	4.73
Heptaglycine	2.94	..	7.69	..	..

Branch and Miyamoto, THIS JOURNAL, **52**, 863 (1930): 2.59,[a] 9.72[d]; 3.12,[c] 8.36[b]; Harned and Owen, *ibid.*, **52**, 5091 (1930): 2.31[a] (25°), 9.75[b] (25°); Edsall and Blanchard, *ibid.*, **55**, 2337 (1933): 2.31[a] (25°), 9.72[b] (25°); 3.14[c] (25°), 8.07[d] (25°); 7.80[m]; 7.75[n] (25°). Owen, *ibid.*, **56**, 24 (1934): 2.37,[a] 9.91[b]; 2.35[a] (25°), 9.78[b] (25°). Neuberger, *Proc. Roy. Soc.*, **158A**, 68 (1927): 3.08[c] (25°), 8.26[d] (25°), 7.71[n] (25°). Stiasny and Scotti, *Ber.*, **63**, 2977 (1930): 2.42,[a] 9.70[b]; 3.13,[c] 8.20[d]; 3.00,[e] 8.00[f]; 3.05,[g] 7.75[h]; 3.05,[i] 7.70[j]; 3.05,[k] 7.60[l] (temp. not recorded). Emerson and Kirk, *J. Biol. Chem.*, **87**, 597 (1930): 7.73[m] (25°).

(20) Ebert, *Z. physik. Chem.*, **121**, 385 (1926); Edsall and Blanchard, THIS JOURNAL, **55**, 2337 (1933).

Discussion

Were it not for the charge on the $-COO^-$ group, pK for the ammonium ion acid $^+NH_3RCOO^-$ would be very similar to that for $^+NH_3RCOOH$ or $^+NH_3RCOOMe$, the value for the latter being about 7.8. The influence of the negatively charged radical is clearly to prevent the removal of the proton from the ammonium ion, and so $^+NH_3RCOO^-$ is a weaker acid than $^+NH_3RCOOH$; pK_C is thus correspondingly greater than pK_B. The difference is largest for glycine itself, as is to be anticipated from the close proximity of the $^+NH_3-$ and $-COO^-$ groups. In diglycine the separation between the charged groups is increased, and hence pK_C falls sharply to 8.23, which is, nevertheless, still appreciably larger than the pK to be expected for the analogous acid without the negatively charged radical. On adding further glycine residues, the value of pK_C falls slowly, up to and including pentaglycine[21]; this is in agreement with the behavior generally observed when the distance between any substituent group and the acidic group in a fatty acid is gradually increased. In view of these facts, it is unexpected to find that there is a further appreciable fall of pK_C in passing from penta- to hexa-glycine, the value being now less than pK_B. It is true that the results of Stiasny and Scotti[9] show no such break, but their experimental procedure is open to criticism, as seen above. The pK_C for hexaglycine given in Table IV has been confirmed by repeating the measurements after further purification of the amino-acid, and the correctness of the results may be regarded as receiving support from the similar value obtained for heptaglycine. It would appear, therefore, at first sight, that the distance between the polar end-groups, which has been increasing slowly in the series di-, tri-, tetra- and penta-glycine, undergoes a sudden increase in hexa-glycine and is maintained in the hepta-compound. This explanation would imply that there is some restriction in the structures of the earlier members of the series which becomes partly or wholly removed in hexaglycine, so that a marked elongation of the molecule is possible. It must be pointed out, however, that the separation of the $-NH_3^+$ and $-COO^-$ groups in

(21) Neuberger, ref. (16), and Westheimer and Shookhoff, ref. (7) calculated the distances between the charged groups in glycine and diglycine from the differences between pK_C and pK_B. For the higher peptides these differences are so small, in comparison with experimental errors and the approximations involved, that the calculations would be valueless.

pentaglycine is already so large that a further increase in the distance is hardly likely to have any appreciable effect on the acidic or basic strength.

The dissociation constant of an acid or base is related to the decrease of free energy of the dissociation process, and hence it is possible for the pK value to change as a result of a marked entropy change. If the ionization process

$$^+NH_3RCOO^- + H_2O \rightleftarrows H_3O^+ + NH_2RCOO^-$$

is accompanied by a structural change leading to an increase of entropy, that is to say, if the dual ion $^+NH_3RCOO^-$ has a restricted structure whereas that of the negative ion NH_2RCOO^- is free, the dissociation constant of the ammonium ion acid will be greater, and hence pK_C will be smaller, than would be the case if they had the same configuration. A possible interpretation of the results obtained in the present work is, therefore, as follows. In the first five members of the glycine series $^+NH_3RCOO^-$ and NH_2RCOO^- have the same unrestricted structure, but in hexaglycine there is a change; the negative ion has the same configuration as previous members of the series, but that of the dual ion has become restricted. This structural change in the $^+NH_3RCOO^-$ ion is presumably maintained in heptaglycine.

Some confirmation of this suggestion can be obtained from the dielectric increments, $\Delta\epsilon/\Delta c$, in aqueous solutions obtained by Wyman[5]; the results for a series of polyglycines, together with the number (n) of glycine residues in the molecule are given below.[22] The differences between the dielectric increments for successive members of the series are also recorded.

n	1	2	3	4	5	6
$\Delta\epsilon/\Delta c$	23	70	113	159	215	234
Difference		47	43	46	56	19

It is seen that the difference between $\Delta\epsilon/\Delta c$ for successive polyglycines is at first almost constant at 46, increases to 56 between tetra- and pentaglycines, and then falls to 19 between the penta- and hexa-compounds. It is possible, on account of the low solubility, that $\Delta\epsilon/\Delta c$ for pentaglycine may be slightly in error; if it is decreased to 205, so as to make the difference between this and the value for tetraglycine equal to 46, $\Delta\epsilon/\Delta c$ for hexaglycine still appears abnormally low. It has been shown[5b] that the $\Delta\epsilon/\Delta c$ values are approximately proportional to the square of the dipole moment, and hence to the square of the mean distance between the charged groups in the dipolar amino-acid molecule. The mean square distance between the ends of a chain, in which there is little or no restriction to rotation, is approximately proportional to the number of atoms in the chain,[23] and hence under these conditions the dielectric increment in the series of glycines may be expected to vary in a linear manner with the number of glycine residues. That it does so up to the pentaglycine suggests that in all these cases there is relatively free movement within the molecule of amino-acid, in spite of the electrostatic attraction between the end-groups. The conclusions are so far in general agreement with those reached from the pK_C values, and the results indicate that in aqueous solution, at least, there is no marked change in configuration in the series from glycine to pentaglycine. The low value of $\Delta\epsilon/\Delta c$ for hexaglycine implies a shorter distance between the charged end-groups than would be the case if there were free movement; hence it appears that when the molecule contains six glycine units there is some modification of the structure of the dual ion which makes free movement more difficult. The mutual attraction of the charges presumably results in a "tied-up" structure of the dipolar ion in this instance.

Attention must now be turned to the pK_A values for the carboxylic acids $^+NH_3RCOOH$; the pK for the uncharged acid NH_2RCOOH is pK_D, but the presence of the positive charge should result in an increase of acid strength, as actually observed. This is most marked for glycine, and the effect diminishes as the distance between the terminal groups is steadily increased. It will be noted from Table IV that there is relatively little change in pK_A in passing from penta- to hexaglycine; it is probable, therefore, that in this substance $^+NH_3RCOOH$ has the same structure as has the dipolar ion $^+NH_3RCOO^-$. If this were not so, then pK_A for hexaglycine would be appreciably greater than the observed value of 3.11. The result for heptaglycine, 2.94, if not due to experimental error, is difficult to explain, although it is important to note that pK_A and pK_D are becoming almost identical, as they must do in the limit.

According to the above discussion, the forms

(22) The results for heptaglycine, recorded as 290 ± 25, is not included, as it was obtained in 5.14 M urea solution on account of the sparing solubility of the acid in water.

(23) Eyring, *Phys. Rev.*, **39**, 746 (1932); Kuhn, *Kolloid-Z.*, **68**, 2 (1934); *cf.* Wyman, *J. Phys. Chem.*, **43**, 143 (1939); see also, Kirkwood and Westheimer, *ibid.*, **6**, 506 (1939), footnote 8, p. 510,

$^+NH_3RCOO^-$ and NH_2RCOO^- of hexa- and hepta-glycine have different structures in solution, whereas those of $^+NH_3RCOO^-$ and $^+NH_3RCOOH$ are the same. The difference in configuration of the positive and negative ions is presumably to be explained by the greater attraction between $—NH_3^+$ and ^-COOH than between $—NH_2$ and $—COO^-$.

It will be observed from Table IV that in spite of the changes in pK_A and pK_C, the dissociation constant of the ammonium ion acid, $^+NH_3RCOOH$, *i. e.*, pK_B, assumed to be equal to the acidic dissociation constant of the ester hydrochloride $^+NH_3RCOOMe$ (or Et), undergoes little change as the number of glycine residues in the molecule is increased. In the case of glycine, $^+NH_3CH_2COOH$, the value of pK_B is determined mainly by the influence of the COOH group, and since this is appreciably further away in diglycine, an increase of pK_B might be anticipated. It must be remembered, however, that the —CONH— group, which is at about the same distance from $^+NH_3$— as the —COOH group is in glycine, will have very much the same influence as the latter group on the strength of the ammonium ion acid.[24] The dissociation constant of the latter may thus not be appreciably altered in the series of glycines, provided the conjugate acid and base, *viz.*, $^+NH_3RCOOH$ and NH_2RCOOH, have the same structure. It is important to note that the values of pK_C in Table IV provide no information on this point; the results were obtained with the esters, and there is no certainty that the corresponding carboxylic acids will behave in an analogous manner.

The value of pK_Z gives an indication of the proportion of dipolar ions to uncharged molecules in a solution of the free amino-acid: this proportion is seen to decrease somewhat in passing from glycine to diglycine, and then to remain almost constant. In view, however, of the uncertainty concerning pK_B for hexaglycine, because of a possible difference in structure between the acid and ester, the value of pK_Z for this acid may be in error.

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(24) This view is borne out by the pK value of 7.93 for glycine amide at 25° (Zief and Edsall, THIS JOURNAL, **59**, 2245 (1937)).

Summary

1. From an examination of the properties of the simpler polyglycines it is concluded that structural changes occur at certain points in the series.

2. The dissociation constants of mono-, di-, tri-, tetra-, penta-, hexa-, and hepta-glycine, of mono-, di-, tri-, and tetra-glycine ethyl ester hydrochloride, and of hexa-glycine methyl ester hydrochloride have been determined at 20° by potentiometric titration using the glass electrode.

3. The results are in general agreement with the concept of a freely rotating chain of increasing length up to and including penta-glycine. The sudden increase in the dissociation constant of the ammonium ion acid $^+NH_3RCOO^-$ at hexa-glycine combined with the low dielectric increment observed by Wyman, suggests that in hexa- and hepta-glycine there is some restriction to free movement in the $^+NH_3RCOO^-$ form which does not exist in NH_2RCOO^-.

4. From the dissociation constants of the ammonium ion acids $^+NH_3RCOOH$, it is concluded that in hexa- and hepta-glycines $^+NH_3RCOOH$ has the same structure as $^+NH_3RCOO^-$.

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