that against x^2 or x_0^2 should be straight (equations 2 and 3), deductions from the latter plot are taken as correct. This would indicate that the rotor had cooled by 0.9° during acceleration from rest to 59,780 r.p.m.—this value of 0.9° depending on the external thermocouple readings. As a final check, the thermodynamically calculated temperatures were plotted on the same graph in Fig. 2 (full-inked circles), and these lie on a straight line approximately 0.88° below the interpolated thermocouple temperatures, the precision of this value again being limited by our ability to read temperatures with the external thermocouple.

Conclusions and Summary

The data in Tables I and II, obtained by a completely independent physical method, are felt to verify the reversible temperature effect discovered by Waugh and Yphantis. The extrapolation and the comparison of thermocouple temperatures with calculated thermodynamic temperatures discussed in the previous section, lend this further verification. We therefore suggest that all previous published Spinco data on sedimentation be corrected to account for this effect. Further, we suggest that the thermocouple calibrations of the oil-turbine ultracentrifuge be re-examined and, if necessary, new calibrations be made, exercising care that only sufficiently thick layers of liquid are used for the extrapolation. It is worthy to note that our method could be employed to advantage in these calibrations, since cell temperatures can be calculated from each photograph and compared directly with the thermocouple temperatures.

Acknowledgment.—This research has been made possible by Grant G3449 of the National Institutes of Health, U. S. Public Health Service, for a study of the sedimentation and diffusion of low molecular weight substances.

WORCESTER, MASS.

[CONTRIBUTION FROM FRICK CHEMICAL LABORATORY, AND THE LOS ALAMOS SCIENTIFIC LABORATORY, UNIVERSITY OF CALIFORNIA]

Physicochemical Studies of the Simpler Polypeptides. III. The Acid- and Base-catalyzed Hydrolysis of Di-, Tri-, Tetra-, Penta- and Hexaglycine

BY EDWARD F. HAMMEL, JR.,[1] AND SAMUEL GLASSTONE[2]

RECEIVED JANUARY 28, 1954

The specific rates for the acid (2 *N* HCl at 65 and 75°) and base (2 *N* NaOH at 20 and 30°) hydrolysis of di-, tri-, tetra-, penta- and hexaglycine have been determined. It is shown that for the tetra- and higher polyglycines these rates increase approximately linearly with the number of glycine residues in the chain. The specific rate for the fission of the central peptide bond in tetraglycine has been derived from the data. Heats and entropies of activation for the acid and base catalyzed hydrolysis of diglycine are reported.

Several studies have been made of the hydrolysis of the simpler polypeptides,[3] but the only systematic investigation of a series of polyglycines was that made by Abderhalden and Suzuki.[3a] These workers reported their results in the form of segmented curves and made no mention of their experimental procedure or of the method used for analyzing the hydrolysate. It may be noted that in a previous paper,[4] dealing with the hydrolysis of other polypeptides, both the Van Slyke[5] and Sörensen[6] methods for the determination of amino acids were employed. The former, however, yields accurate results with polyglycines only when the modification of Kendrick and Hanke,[7] which was not published at the time, is employed, and in the experience of the present authors the Sörensen titration is not reliable unless carried out potentiometrically. An examination of Abderhalden's curves revealed two marked discrepancies: In the first place, the rate of hydrolysis of hexaglycine in acid solution appeared to be little more than twice as great as that of diglycine whereas a much larger ratio is to be expected. and, in the second place, the heats of activation for both acid and alkaline hydrolysis were calculated to lie between 3.5 and 5.5 kcal. in all cases but one. It is improbable that the reactions under consideration will have such low activation energies, and this view is confirmed by values of the order of 15 kcal. found in connection with the hydrolysis of esters, a process quite analogous to that involved in the splitting of polypeptides.

The foregoing results are probably to be attributed to difficulties in the analysis of the mixtures of glycine and polyglycines obtained in the course of the hydrolytic reaction. The unexpected observations reported by Yaichnikov and Spiridonova[8] are probably due to the same cause. They reported that the acid (1 *N* HCl) hydrolysis of diglycine (0.1 *N*) is complete in 30 hours at 95° but subsequently there is a decrease of amino nitrogen, suggesting a recombination, cyclization or some other reaction which causes glycine to disappear. Repe-

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(3) (a) E. Abderhalden and H. Suzuki, *Z. physiol. Chem.*, **170**, 158 (1927); **173**, 250 (1928); (b) W. Kuhn, C. C. Molster and K. Freudenberg, *Ber.*, **65B**, 1179 (1922); (c) P. A. Levene, *J. Biol. Chem.*, **82**, 167 (1929); (d) L. Lawrence and W. J. Moore, THIS JOURNAL, **73**, 3793 (1951).

(4) E. Abderhalden and H. Sickel, *Z. physiol. Chem.*, **170**, 134 (1927).

(5) D. D. Van Slyke, *J. Biol. Chem.*, **83**, 425 (1929).

(6) S. P. L. Sörensen, *Biochem. Z.*, **7**, 45 (1907).

(7) A. B. Kendrick and M. E. Hanke, *J. Biol. Chem.*, **117**, 161 (1937).

(8) I. S. Yaichnikov and A. S. Spiridonova, *J. Gen. Chem. (USSR)*, **4**, 1286 (1934).

tition of this experiment by the present authors under slightly different conditions did not confirm these results.

In the work to be described in this paper a considerable gain in analytical accuracy was achieved by the use of an improved method of separating the hydrolysate from the acid or alkaline catalyst, and the end-points were greatly improved by carrying out the Sörensen titration potentiometrically with a glass electrode.

Experimental

Procedure.—The polyglycines were prepared by the methods described in an earlier paper,[9] the purity of each compound being checked by a potentiometric formol titration, with the following results: diglycine 99.2%, triglycine 97.5%, tetraglycine 98.8%, pentaglycine 96.2%, hexaglycine 94.7%. The reaction vessel consisted of a three-necked flask fitted with ground-glass joints: the center opening carried a mercury-sealed stirrer, the second, a condenser and thermometer, and the third, a sampling device. The latter consisted of a capillary tube extending from the bottom of the reaction flask through a three-way stopcock to a water-jacketed 5-ml. micro-buret kept at 20°. To remove a sample of reaction mixture for analysis, the buret was filled by suction, and a definite volume, measured at 20°, was run off through the third outlet of the stopcock. The reaction flask was maintained at constant temperature in a thermostat which was controlled to ±0.03°.

A measured volume of 2 *N* hydrochloric acid or 2 *N* sodium hydroxide was introduced into the flask. When this reached the temperature of the thermostat a weighed amount of the finely powdered polypeptide, sufficient to make the solution 0.025 *N*, was added. After stirring rapidly for 30 seconds, solution was complete, and a sample was immediately withdrawn and analyzed. This was taken as the solution concentration at zero time. Further samples were withdrawn from time to time during the course of the experiment and analyzed in the manner described below. In the acid hydrolysis the cooling to 20° in the measuring buret was sufficient to "quench" the reaction. With the alkaline catalyst, however, the hydrolysis was much more rapid; consequently, the alkaline reaction mixture was "quenched" by mixing it (immediately after its volume had been measured) with a slight excess of solidified 2 *N* hydrochloric acid at −78°. This cooled the sample to a temperature at which further hydrolysis was negligible and stabilized the mixture with respect to further hydrolysis.

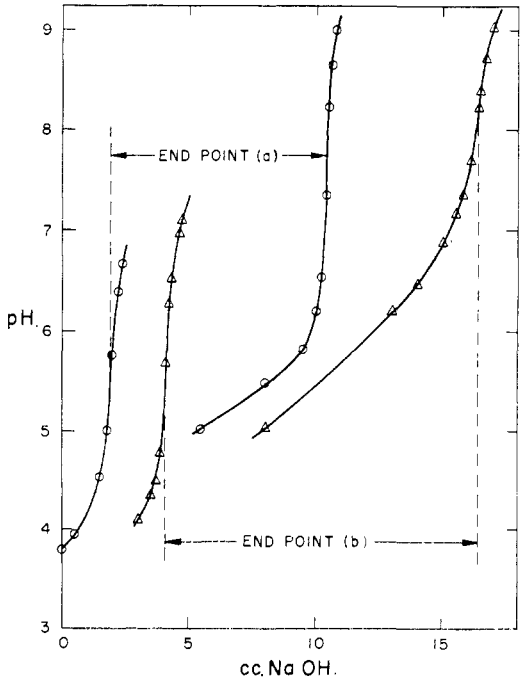

Fig. 1.—Typical titration curves of polyglycine hydrolysates. Curves represent formol titrations of tetraglycine with 0.01034 *N* NaOH at zero time (a), and after 210 minutes (b) for acid-catalyzed hydrolysis at 75°.

(9) S. Glasstone and E. F. Hammel, THIS JOURNAL, **63**, 243 (1941).

Analysis.—Since the hydrochlorides of glycine and the polyglycines can be titrated with alkali to give sharp end-points, attempts were made in the study of the acid-catalyzed hydrolysis to remove quantitatively excess acid from the samples withdrawn from the reaction mixture, leaving the hydrochlorides in the pure state. However, efforts to precipitate the pure salts by means of various non-aqueous solvents or to remove excess of acid by evaporation from these solvents at low temperature proved unsuccessful,[10] and the following alternative method of analysis had to be employed.

The acidified reaction mixture was evaporated to dryness at 3 to 5° *in vacuo;* this served to remove most of the free acid, and the remainder plus a fraction of the peptide hydrochlorides was neutralized by a drop or two of *N* sodium hydroxide. The solution of the residue was titrated potentiometrically with 0.025 *N* sodium hydroxide until a rapid change of potential of the glass electrode indicated that the isoelectric state of the system was attained. A quantity of neutralized formalin, sufficient to make the solution contain approximately 12% of formaldehyde, was then added and the titration continued to the second end-point. The value of this titration gave the number of moles of amino groups present in the reaction mixture at the time the sample was withdrawn. Figure 1 illustrates the typical variation of *p*H with addition of standard base for a mixture of polyglycines.

Calculation.—The hydrolysis of a polyglycine may be regarded as involving both simultaneous and consecutive reactions. A simplification of the involved calculation of the specific rates was made possible by expressing the data in terms of the number of unbroken peptide linkages rather than of the concentrations of the successive individual polyglycines. The number of moles (B) of peptide bonds existing in a solution originally of concentration G^0_n after a fraction x of the total original numbers of bonds has been split is given by [11]

$$B = (n - 1)G^0_n(1 - x) \qquad (1)$$

For every mole of peptide bonds broken there is obtained an additional mole of amino groups; hence if T is the total number of moles of amino groups at any time, t, then

$$T = G^0_n + x(n - 1)G^0_n \qquad (2)$$

Combination of 1 and 2 gives

$$B = nG^0_n - T \qquad (3)$$

In terms of the products of hydrolysis, the number of unbroken peptide bonds at any instant can be written as

$$B = (n - 1)G_n + (n - 2)G_{n-1} + \ldots \qquad (4)$$

where G_n, G_{n-1}, etc., are the concentrations of the particular polyglycines after time, t; hence from 3 and 4

$$nG^0_n - T = (n - 1)G_n + (n - 2)G_{n-1} + \ldots \qquad (5)$$

The values of G_n, G_{n-1}, etc., can be obtained in the usual manner by the integration of the appropriate differential rate equations, as indicated in the previous paper,[11] utilizing the results of Bateman.[12] In general, it is possible to write G_n, G_{n-1}, etc., as a product of G^0_n for the appropriate polyglycine and a function of the specific rate constants, k_n, k_{n-1} . . ., and the time, t. Thus equation 5 becomes

$$\frac{nG^0_n - T}{G^0_n} = (n - 1)f_n(k_n, k_{n-1}, t) + (n - 2)f_{n-1}(k_{n-1}, k_{n-2}, t) + \ldots \qquad (6)$$

where f_n, f_{n-1}, f_{n-2}, ... etc., represent functions of the specific reaction rates and the time. The left hand side of

(10) *Cf.* J. Mika, *Mikrochem., Molisch Festschrift*, 319 (1936).

(11) For general notation, see Glasstone and Hammel, THIS JOURNAL, **63**, 2003 (1941). The authors are indebted to Professor John Tukey of the Department of Mathematics, Princeton University, for suggestions on the following kinetic calculations.

(12) H. Bateman, *Proc. Cambridge Phil. Soc.*, **15**, 423 (1910).

6 can be obtained directly from the initial titration of the reaction system, which is proportional to G^0_n, and that at time, t, which is proportional to T. Starting with diglycine (G_2), the value of the corresponding specific rate of hydrolysis (k_2) is obtained directly from the usual equation for a first-order reaction. Knowing k_2, it is possible to determine k_3 from experiments on triglycine (G_3) and the appropriate form of equation 6. In general k_n can be derived from measurements on the polyglycine G_n, provided k_{n-1}, k_{n-2}, ..., k_2 are known from experiments on G_{n-1}, G_{n-2}, ..., G_2.

The foregoing method of calculating the specific reaction rates presupposes that each polyglycine is split up in one way (or in equivalent ways), and that there are no alternative reactions. This is true for diglycine and triglycine, for although the latter has two peptide linkages, the occurrence of hydrolysis at either of them will yield the same products, diglycine and glycine. With tetraglycine, however, the method fails. While it is true that fission at either of the terminal peptide linkages will produce triglycine and glycine, cleavage of the middle linkage yields two molecules of diglycine. We therefore modify the above procedure as follows:

Let the specific rates for hydrolysis at the various –NH–CO–linkages be designated by means of the superscripts $\alpha, \beta, \gamma, \ldots$, where α refers to the linkage nearest the formal charge on the polypeptide molecule, *i.e.*, $-NH_3^+$ in acid and $-COO^-$ in alkaline solution, β to the next nearest, etc., and ω to the other terminal linkage. Then for tetraglycine, the kinetic equations are

$$-dG_4/dt = k_4G_4;\quad dG_3/dt = (k_4^\alpha + k_4^\omega)G_4 - k_3G_3;$$

$$dG_2/dt = 2k_4^\beta G_4 + k_3G_3 - k_2G_2$$

the over-all specific rate k_4 is the sum of the individual values for the separate linkages k_4^α, k_4^β and k_4^ω. Integration of these equations gives (after extracting G^0_n) the values of f_n, f_{n-1}, etc., for insertion in equation 6. Taking the sum $k_4^\alpha + k_4^\omega$ as one variable, there still remains k_4^β. The latter may be expressed in terms of k_4 and $k_4^\alpha + k_4^\omega$, since $k_4 = k_4^\alpha + k_4^\beta + k_4^\omega$. Then k_4 may be found independently in the following manner. In the initial stage of any consecutive reaction the subsequent stages may be neglected. For the hydrolysis of polyglycines differentiation of equation 5 gives

$$k_nG^0_n = (dT/dt)_{t=0} \qquad (7)$$

The value of the right-hand side may be obtained graphically from the plot of the titrations against time, and in this way k_n is independently determined.

Although this procedure provides, in principle, a method for obtaining $k_4^\alpha + k_4^\omega$ and k_4^β from the measurements on tetraglycine, the specific rates which were originally calculated from the experimental data were not satisfactory (for example, some were negative) and were therefore not published.

Recently, however, the data were reanalyzed at the Los Alamos Scientific Laboratory with the aid of an IBM automatic computing machine. It was found that the constancy of the specific rate calculated for k_4^β was quite sensitive to the choice of k_4 as obtained from the initial slope. Variation of only a few per cent. in k_4, within the limits of the experimental error of that originally chosen as the "best" value, caused k_4^β to change sign! From the IBM analysis it was possible to choose self-consistent values of k^4 and k_4^β from the titration data for the different temperatures.

Unfortunately similar analysis of the remaining data for penta- and hexaglycine does not appear profitable. As a consequence of the original inability to obtain constant values for the specific rates of the individual peptide linkages, the experimental data for penta- and hexaglycine emphasized the initial portion of the hydrolysis. In this way satisfactory values for the over-all rate constants could be obtained in most cases by means of equation 7, but there are insufficient data for an IBM type analysis. Even for tetraglycine, the data for acid hydrolysis at 65° and alkaline hydrolysis at 30° are rather unsatisfactory for the same reason. In addition decomposition of the higher peptides in alkaline solution apparently occurs as shown by the lack of constancy of the specific rates for the alkaline hydrolysis of triglycine (see Table I). Similar difficulties were reported by Lawrence and Moore[3d] and others.[13]

Results and Discussion

The experimental results for acid (2 *N* HCl) and alkaline (2 *N* NaOH) hydrolysis of five polyglycines, each at two temperatures, are summarized in Table I. The specific rates have been computed both analytically, from equation 6, and graphically, from equation 7, and are tabulated separately as indicated. Specific rates listed opposite a zero time entry are extrapolated values and presumably the most reliable. The experimental data for acid hydrolysis at 75° for the series of polyglycines are presented in a reduced form in Fig. 2. In the case of basic hydrolysis, especially at 30°, curvature of the T *vs.* time curves in the vicinity of $t = 0$ is so large for penta- and hexaglycine that reliable values of k_5 and k_6 cannot be obtained from the data. The values given in Table I for these rate constants are uncertain to 20–30% and are included for order of magnitude purposes only. The results of the IBM calculations are given in the footnotes.

TABLE I

ACID HYDROLYSIS

	65°			75°		
Peptide	Time, min.	k_n (min.$^{-1}$) × 10^2 from eq. 6	k_n (min.$^{-1}$) × 10^2 from graph, eq. 7	Time, min.	k_n (min.$^{-1}$) × 10^2 from eq. 6	k_n (min.$^{-1}$) × 10^2 from graph, eq. 7
Diglycine	0	0.0453	0.045	0	0.109	0.11
	242	.0458		60	.110	
	660	.0467		150	.112	
	1320	.0481		225	.114	
				360	.117	
Triglycine	0	.247	.25	0	.560	0.56
	150	.252		60	.562	
	420	.261		180	.567	
	1080	.284		360	.574	
				590	.583	
Tetraglycine		.723 (IBM)[a]	.70		1.36 (IBM)	1.3
Pentaglycine		..	.99		..	1.7
Hexaglycine		..	1.24		..	2.3

ALKALINE HYDROLYSIS

	20°			30°		
Peptide	Time, min.	k_n (min.$^{-1}$) × 10^2 from eq. 6	k_n (min.$^{-1}$) × 10^2 from graph, eq. 7	Time, min.	k_n (min.$^{-1}$) × 10^2 from eq. 6	k_n (min.$^{-1}$) × 10^2 from graph, eq. 7
Diglycine	0	0.246	0.25	0	0.575	0.58
	120	.244		90	.582	
	240	.249		210	.562	
	360	.245		360	.580	
	540	.245				
Triglycine	0	.98	1.0	0	2.1	2.1
	75	.923		60	2.0	
	212	.81		165	1.3	
	330	.73		240	1.1	
	540	.52				
Tetraglycine		2.6 (IBM)[c]	2.7		6.7 (IBM)	6.7
Pentaglycine			3.6			8.6
Hexaglycine			5.3			12.7

[a] IBM data give: k_4^β(65° acid hydrolysis) = $0.2 - 0.3 \times 10^{-2}$. [b] IBM data give: K_4^β (75° acid hydrolysis) = 0.4×10^{-2}. [c] IBM data give: K_4^β (20° base hydrolysis) = 1.8×10^{-2}. This compares favorably with Kuhn, Molster and Freudenberg data, ref. 3b.

(13) See D. French and J. T. Edsall, *Adv. in Protein Chem.*, **II**, 301 (1945).

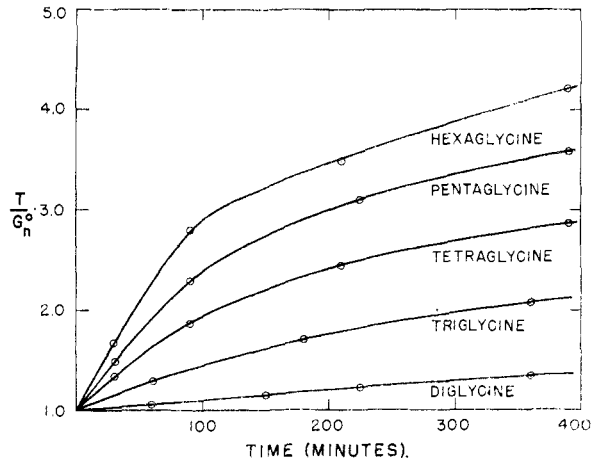

Fig. 2.—Acid-catalyzed hydrolysis of the polyglycines at 75°.

The heat and entropy of activation for the acid- and alkaline-catalyzed hydrolysis of diglycine are given in Table II.

The reactions occurring during the hydrolysis of a polyglycine consist of a combination of both simultaneous and consecutive processes, the over-all specific rate for a polyglycine molecule containing n glycine residues being written, in the most general case, as

$$k_n = k\alpha_n + k\beta_n + k\gamma_n + \ldots\ldots + k\omega_n \qquad (8)$$

Since internal peptide linkages sufficiently remote from the terminal groups will be subjected to almost identical inductive and electrostatic forces and will hence have equal rate constants, the increments in over-all specific rate observed in passing from one polyglycine to the next higher in the series should eventually become equal. Such a relationship was in fact predicted by Kuhn, Molster and Freudenberg[3b] who suggested that the rate of hydrolysis of a polyglycine consisting of n glycine residues ($n > 4$) could be represented by

$$k_n = (k_4^{\alpha} + k_4^{\omega}) + (n - 3)k_4^{\beta} \qquad (9)$$

From Figs. 3 and 4 it will be seen that this expression appears to be approximately correct. Equation 9 implies, however, in the opinion of the present authors, an over-simplification of the complicated electrostatic and inductive effects which determine the specific rate constants for the individual peptide bonds. It will be seen from Figs. 3 and 4 that the total specific reaction rate for tetraglycine lies in every case above the curve, indicating that the increment in specific rate in passing from tri- to tetraglycine is greater than the average value predicted by equation 9. Furthermore, heats and entropies of activation calculated for an "internal" peptide bond from the slopes of the curves given in Figs. 3 and 4 give values which appear too low in the case of acid hydrolysis and too high in the case of alkaline hydrolysis. Nevertheless the basic idea of Kuhn, Molster and Freudenberg appears sound, and although there is some evidence from the present work for believing that the constancy in the rate for internal linkage splitting appears after tetraglycine rather than after triglycine, as postulated by Kuhn, *et al.*, more precise measurements of the hydrolysis of still higher polyglycines will be required to establish this point definitely.

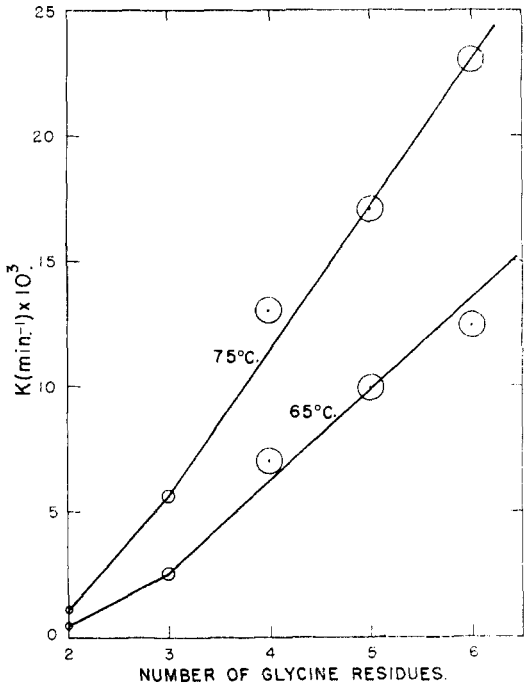

Fig. 3.—Variation of specific rate with number of glycine residues—acid hydrolysis.

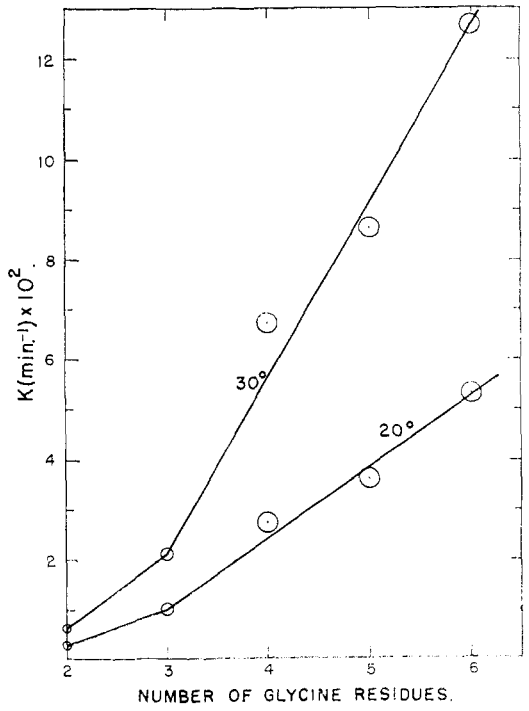

Fig. 4.—Variation of specific rate with number of glycine residues—alkaline hydrolysis.

The heat and entropy of activation may be computed from the data given in Table I for the acid- and base-catalyzed hydrolysis of diglycine. For triglycine it is impossible to obtain the specific rates for the individual peptide bonds from kinetic

data alone. Hence the heat and entropy of activation have not been computed for this peptide. Similarly for higher peptides, heats and entropies of activation are meaningless unless related to the scission of a specific peptide bond and are therefore not reported. The results for the heat and entropy of activation for diglycine are given in Table II together with similar data from other investigations.

TABLE II

HEAT AND ENTROPY OF ACTIVATION FOR ACID- AND BASE-CATALYZED HYDROLYSIS OF DIGLYCINE

	Acid hydrolysis	Base hydrolysis
$\Delta H^{\pm}$ (kcal./mole)	19.1	14.1
	20.3[a]	14.9[b]
		16.9[a]
$\Delta S^{\pm}$ (e.u.)	−30.4	−35
	−24.0[a]	−26.8[a]

[a] Ref. 3d. [b] Ref. 3b.

It will be seen that the entropy of activation is large and negative in both acid- and base-catalyzed hydrolysis due to the low probability of formation of an activated complex from two ions (the peptide and the catalyst) and a water molecule. The difference of about 5 kcal./mole in activation energy between acid and base hydrolysis may be related to the fact that in alkaline hydrolysis the formation of the activated complex is favored by the attraction of the negative hydroxyl ion to the positive carbonyl carbon in the peptide bond, whereas in acid hydrolysis the H_3O^+ ion must oppose the positively charged nitrogen atom. This situation is quite analogous to the similarly catalyzed ester hydrolysis.[14]

Acknowledgment.—The authors gratefully acknowledge a grant from the American Philosophical Society for partial support of this research.

(14) S. Glasstone, K. J. Laidler and H. Eyring, "Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, p. 451.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Diffusion in Sugar Solutions. III. Diffusion of Sucrose in Glucose Solutions as Solvent

BY DAVID M. CLARKE AND MALCOLM DOLE

RECEIVED AUGUST 3, 1953

Diffusion experiments have been performed by the Gouy interference method in mixed water–glucose–sucrose systems with sucrose present in small concentrations. Diffusion coefficients of sucrose at infinite dilution in the glucose solutions were calculated by the height–area approximation method. It is shown that independent diffusion of the two solutes does not occur, that large discrepancies exist between the height–area and weight–average diffusion coefficients, that diffusion between two solutions having equal vapor pressures of water approximates the ideal Gaussian pattern, and that the diffusion coefficient of sucrose calculated from the latter experiment agrees well with the data from the other less ideal experiments. The diffusion coefficient of sucrose is greater in the glucose solutions when compared at the same total mole fraction of sugar than in its own solutions. The activation energy for diffusion approaches that for glucose diffusion. A method of performing diffusion experiments on multiple component systems so that the measured average diffusion coefficient will equal that of one component is suggested.

I. Introduction

This paper is the third in a series devoted to the study of diffusion in sugar solutions.[1] English and Dole[2] studied sucrose solutions and Gladden and Dole,[3] glucose solutions, all in water as the solvent. The present paper extends the work to the three-component system, water–glucose–sucrose, with the sucrose–glucose ratio in most solutions being very small.

If metals are left out of consideration, investigations on diffusion in three-component systems[4] have not been extensive. However, the new optical methods for the study of diffusion have stimulated efforts to determine diffusion coefficients in mixtures, particularly among the biochemists.[5]

Ogston[6] was apparently the first to apply the Gouy interference method[7–9] to mixtures; he studied the diffusion of lactoglobulin in solutions which had been dialyzed against a sodium acetate–acetic acid buffer and to which weighed amounts of sucrose or potassium chloride had been added. Using a rather complicated method of analyzing his data, he obtained good agreement to about 2% between the observed diffusion coefficients of both solutes and their known values.

Gralén[10] suggested the use of the ratio between the diffusion coefficient calculated from the second moment and the diffusion coefficient calculated using the zero'th moment, the height–area diffusion coefficient, as a measure of the polydispersity of the solution. Charlwood[11] recently concluded that use of the fourth moment in studying the polydispersity of the solution was not practical, despite its theoretical advantage in yielding the standard deviation (in conjunction with the second moment),

(1) The first two papers were entitled diffusion in "supersaturated" solutions, but as the second and this paper cover the range from low to high concentrations the restricting word "supersaturated" has been omitted from the title.

(2) A. C. English and M. Dole, THIS JOURNAL, **72**, 3261 (1950).

(3) J. K. Gladden and M. Dole, *ibid.*, **75**, 3900 (1953).

(4) The paper of Freundlich and Kruger, *J. Phys. Chem.*, **43**, 981 (1939), on the diffusion of quinone in salt solutions contains references to earlier papers. McBain and co-workers have studied mixtures of ions and ions with glycine or egg albumin: J. W. McBain and C. R. Dawson, THIS JOURNAL, **56**, 52, 1021 (1934); J. R. Vinograd and J. W. McBain, *ibid.*, **63**, 2008 (1941).

(5) H. Neurath, *Chem. Revs.*, **30**, 357 (1942).

(6) A. G. Ogston, *Proc. Roy. Soc.* (*London*), **196A**, 272 (1949); *Biochem. J.*, **45**, 189 (1949).

(7) G. Kegeles and L. J. Gosting, THIS JOURNAL, **69**, 2516 (1947).

(8) L. G. Longsworth, *ibid.*, **69**, 2510 (1947).

(9) L. J. Gosting and M. Morris, *ibid.*, **71**, 1998 (1949).

(10) N. Gralén, *Kolloid Z.*, **95**, 188 (1941).

(11) P. A. Charlwood, *J. Phys. Chem.*, **57**, 125 (1953).