[CONTRIBUTION FROM THE CATHOLIC UNIVERSITY OF AMERICA]

Kinetics of the Hydrolysis of Simple Glycine Peptides

BY LORRAINE LAWRENCE[1] AND WALTER J. MOORE

The kinetics of the hydrolyses in hydrochloric acid of glycylglycine, acetylglycine, DL-alanylglycine, glycyl-L-tyrosine, glycyl-L-leucine, glycyl-L-tryptophan, and L-leucylglycine, have been studied over a range of temperatures. The relative rates at 54.4° are 1, 9.9, 0.56, 0.52, 0.48, 0.44 and 0.18, respectively. Variations in both heats ΔH^* and entropies ΔS^* of activation determine the relative rates. The ΔH^* values range from 19.7 to 21.6 kcal. mole^{-1}, and the ΔS^* from -16.6 to -27.1 cal. deg.$^{-1}$ mole^{-1}. The hydrolysis of glycylglycine catalyzed by an acid resin, Dowex 50, has a $\Delta H^* = 20.1$, and $\Delta S^* = -19.0$; the ΔH^* is slightly lower and the ΔS^* markedly higher than the hydrochloric acid values. Addition of cobaltous ion to the hydrochloric acid medium lowers both ΔH^* and ΔS^*, but the net effect is a 70% enhancement of the rate at 54°. In acid hydrolysis at 54.4° the first peptide bond in diglycylglycine is split 8 times more rapidly than glycylglycine, the effect being mainly due to the entropy factor. The carboxypeptidase-catalyzed hydrolysis of carbobenzoxyglycyl-L-phenylalanine has been investigated at 15° and 25° over a limited range of substrate concentrations. The $\Delta H^* = 10.9$ and $\Delta S^* = -6.4$ indicate that the high catalytic efficiency of the enzyme is due to both a decrease in ΔH^* and an increase in ΔS^*.

Most of the available rate data on peptide hydrolysis are restricted to a single temperature, so that the activation energies and entropies cannot be calculated. Synge[2] followed the hydrolysis of 10 different dipeptides in a 1:1 mixture of 10 *N* hydrochloric acid and glacial acetic acid at 37°, and found in every case that the rate was decreased by substitution of an α-hydrogen in glycine by an alkyl group. Goldschmidt and Füner[3] studied the hydrolysis of benzoylated amino acids and several peptides at 25 and 100°, using either 2 to 8% sodium hydroxide or 40 to 70% sulfuric acid. Kuhn, Molster and Freudenberg[4] measured the sodium hydroxide catalyzed hydrolyses of diglycylglycine at 20° and glycylglycine at 20 and 34°. Escolme and Lewis[5] measured the hydrolyses of acetylglycine and benzoylglycine at 60 and 70° at several hydrochloric acid concentrations.

In addition to their usefulness in studies of protein hydrolysis, data on the hydrolysis rates of different dipeptides should provide an insight into the mechanisms of the hydrolytic reaction. Since the peptides exist in acid solution as positive ions and in basic solution as negative ions, it is not to be expected *a priori* that a very exact model for their kinetic behavior can be provided by amides or N-acylated amino acids. Since hydrolytic enzymes apparently act as acid–base catalysts, some of the results of these studies may be applicable to the problems of enzymatic degradation and synthesis of proteins. A number of experiments were made using the cation exchange resin, Dowex 50 (Nalcite),[6] which may provide a model of a typical solid acid catalyst. Some results will be reported with the enzyme carboxypeptidase, to illustrate how the activation energy is lowered by this typical peptidase.

Experimental

The hydrochloric acid hydrolyses of the following compounds were studied at 55, 70 and 85°: glycylglycine, DL-alanylglycine, glycyl-L-leucine, L-leucylglycine, glycyl-L-tyrosine, glycyl-L-tryptophan, acetylglycine and diglycylglycine. In addition, studies were made of the hydrolysis of glycylglycine catalyzed by Dowex 50, by hydrochloric acid in the presence of cobaltous ion, and by sodium hydroxide, and of the hydrolysis of carbobenzoxyglycyl-L-phenylalanine catalyzed by carboxypeptidase.

Materials.—Glycylglycine and diglycylglycine were synthesized by the chloroacetyl chloride method. They were analyzed by formol titration[7] after several recrystallizations. Molecular weights: glycylglycine, 131.9 obsd., 132.1 calcd.; diglycylglycine, 193.2 obsd., 189.1 calcd. The other peptides were products of the Hoffmann–La Roche Co. Formol titrations yielded: alanylglycine, 143.9 obsd., 146.1 calcd.; glycyltyrosine, 236.6 obsd., 238.2 calcd. The other peptides were not tested by formol titration, but in every kinetic run a determination by the semi-micro ninhydrin method[8] indicated that the free amino acid initially present in the sample was less than 1%, except for diglycylglycine (2.3%). Carbobenzoxyglycyl-L-phenylalanine was synthesized by the carbobenzoxy synthesis[9] and carboxypeptidase (3 × cryst.) was obtained as an aqueous suspension from the Worthington Biochemical Laboratories.

Experimental Procedures.—In following the course of peptide hydrolysis two analytical procedures were considered, the formol titration[7] and the volumetric ninhydrin method.[8] The ninhydrin method was found to be more suitable for two reasons: (1) in the presence of large amounts of hydrochloric acid it is difficult to determine accurately a small increment of acidity due to peptide hydrolysis; (2) in the case of unsymmetrical peptides it is rather difficult to determine accurately the end-point of the formol titration, since the hydrogen-ion concentration in the solution depends on the dissociation constants of the dipeptide and of two different amino acids. In the case of glycylglycine both methods were used and the results were in good agreement even though the end-points became less sharp as the hydrolysis proceeded.

In the acid hydrolysis runs, peptide solutions 0.07 to 0.10 molar were prepared in 0.6 to 0.8 molar aqueous hydrochloric acid. The ionic strength of the solution was adjusted to 2.8 with potassium chloride. Samples of 2 to 3 ml. each were sealed in Pyrex tubes. At each temperature six to eight tubes were taken for a run. The sample tubes were immersed in oil-baths controlled to ±0.1°. At intervals a tube was withdrawn, its tip broken and a 1- or 2-ml. sample pipetted for analysis. Procedure in the basic hydrolysis runs was similar, except that an alkali-resistant glass, Corning no. 728, was used for the reaction tubes; the glycylglycine was 0.05 molar, the sodium hydroxide was 0.21 molar, and the ionic strength was again 2.8. In the runs with added cobaltous ion, the initial substrate concentration was 0.07 molar, the cobaltous ion was added as 0.05 molar cobaltous chloride, and the ionic strength was 2.8.

In the runs with Dowex 50, the glycylglycine was dissolved in triply distilled water to a concentration of 0.15 molar. This solution was added to 0.3- to 0.4-g. samples of resin in separate reaction tubes, 9.00 ml. of solution being added per 1.000 g. of resin. The tubes were cooled during the addition, then sealed and thermostated in oil-baths equipped with

(1) From a dissertation presented by Lorraine Lawrence, in partial fulfillment of the requirements for the Ph.D. degree.

(2) R. L. M. Synge, *Biochem. J.*, **39**, 351 (1945).

(3) S. Goldschmidt and W. Füner, *Ann.*, **483**, 190 (1930).

(4) W. Kuhn, C. C. Molster and K. Freundenberg, *Ber.*, **65**, 1179 (1932); *cf.* Abderhalden, *Z. physiol. Chem.*, **170**, 146, 158 (1927).

(5) A. I. Escolme and W. C. M. Lewis, *Trans. Faraday Soc.*, **23**, 651 (1927).

(6) This material is a sulfonated polystyrene. See W. C. Bauman, J. R. Skidmore and R. H. Osmun, *Ind. Eng. Chem.*, **40**, 1350 (1948).

(7) M. S. Dunn and A. Loshakoff, *J. Biol. Chem.*, **113**, 359 (1936).

(8) D. D. Van Slyke, D. A. MacFayden and P. Hamilton, *ibid.*, **141**, 671 (1941).

(9) K. Hofmann and M. Bergmann, *ibid.*, **134**, 225 (1940).

racks to rotate the tubes end over end at 30 r.p.m. The resin was prepared as follows: It was placed in a column 2.5 cm. in diameter and 80 cm. long, and a liter of 10% HCl solution was slowly passed through it, followed by a liter of 10% NaCl solution. This cycle was repeated twice. A final treatment with 10% HCl was followed by 2 liters of triply distilled water. The effluent water was then virtually free of chloride ion. The resin was dried in air at 80° for 16 hr. A 40 to 60 mesh fraction was screened and further dried for 2 hr. at 80° before use. The acid content of the resin was determined by adding excess sodium chloride to displace the hydrogen ion and titrating with 0.5 *N* sodium hydroxide; the end-point detected with a glass-electrode *p*H-meter is sharp and stable. The resin appears to decompose, forming acid when heated in glycine solutions above 80°, but at 65°, the upper limit for these runs, there is no detectable decomposition in 48 hr. In the runs with resin, the contents of each sample tube were treated with excess sodium chloride and titrated by the formol method using the *p*H-meter. Titrations of known mixtures of glycine, glycylglycine and acid resin showed that this method gives satisfactory results, within 0.2%.

The enzymatic hydrolysis was followed by the ninhydrin procedure. Temperatures of 15.0 and 25.0° were chosen, and the solutions were made in phosphate buffers of *p*H 7.6. A sample was withdrawn from the thermostat and pipetted into a flask containing solid buffer and water which had been cooled to −20°. The sample was brought rapidly to a boil and the ninhydrin analysis continued as usual. Initial blanks showed that no detectable enzymatic hydrolysis occurred before the enzyme was inactivated by heating.

Results

Acid Hydrolysis.—The hydrolysis of a dipeptide in a solution of a strong acid can be represented as

$$^{+}NH_3\cdot CH(R_1)\cdot CO\cdot NH\cdot CH(R_2)\cdot COOH + H_3O^{+} = {}^{+}NH_3\cdot CH(R_1)\cdot COOH + {}^{+}NH_3\cdot CH(R_2)\cdot COOH$$

One H_3O^{+} is consumed for each peptide bond that is split. Unless a large excess of acid is used, it is necessary to represent the rate by a second-order equation. If the initial concentration of dipeptide is a mole liter^{-1}, and that of H_3O^{+} is b, one obtains, writing x for the concentration of dipeptide that is hydrolyzed after a time t

$$k_2t(b - a) = \ln \frac{a(b - x)}{b(a - x)}$$

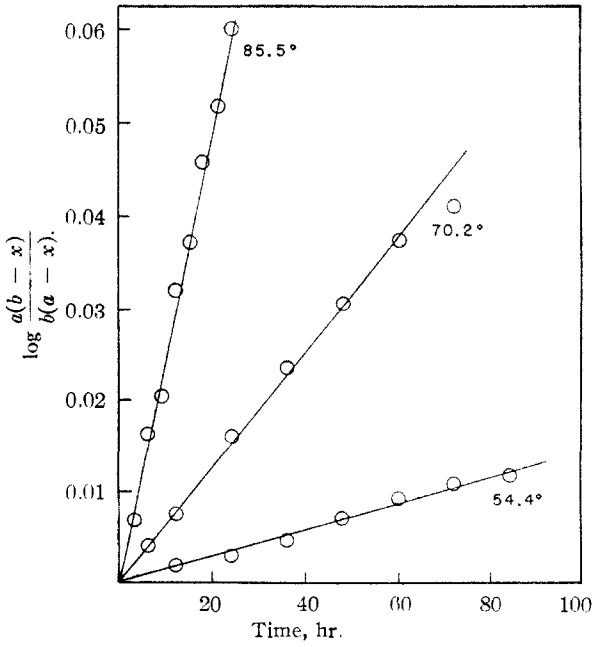

Fig. 1.—The hydrochloric acid hydrolysis of DL-alanylglycine, plotted according to the second-order rate law.

In Fig. 1 are typical data plotted according to this equation. A similar fit is obtained with the other compounds.

The rate constants, calculated from the least-squares straight lines, are summarized in Table I. Included in the table are the values of the heat of activation, ΔH^*, and the entropy of activation, ΔS^*, with their probable errors, calculated by the least-squares method from the equation

$$k_2 = (kT/h)e^{\Delta S^*/R}e^{-\Delta H^*/RT}$$

In Fig. 2 are plots of log (k_2/T) *vs.* $1/T$, from which the ΔH^* and ΔS^* values in the table are obtained.[10]

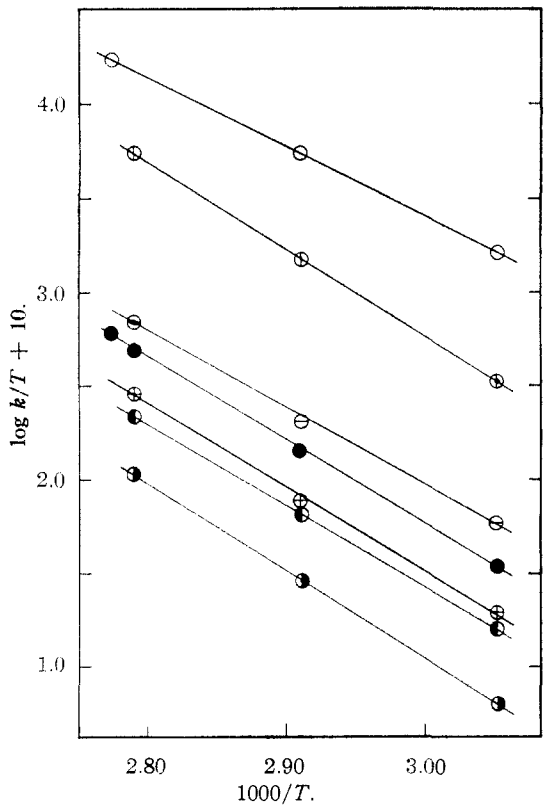

Fig. 2.—Plots of log k/T *vs.* $1/T$ for the hydrochloric acid hydrolyses of peptides, except where noted: ○, basic hydrolysis of glycylglycine; ⊕, acetylglycine; ⊖, glycylglycine, Co^{++}-activated; ●, glycylglycine; ⊕, alanylglycine; ◐, glycylleucine; ◑, leucylglycine. (Glycyltyrosine lies between alanylglycine and glycylleucine; glycyltryptophan is similar to glycylleucine.)

In calculating the rate constant of the reaction with Dowex 50, the hydrogen-ion concentration in the rate expression was replaced by $1000\ n_H m_R/V$. Here n_H is the number of equivalents of hydrogen ion in a gram of resin, m_R the mass of dry resin taken for each run, and V is the volume in milliliters of aqueous solution added to the dry resin. The standard state to which the activation entropies in Table I refer is therefore one equivalent of hydrogen ion per liter of solution. The ionic strength could not be adjusted by the addition of any salt, because the added cations would displace the hydrogen ions. Results of the runs with resin are shown in Fig. 3. When correction is made for hydration

(10) ΔH^* is almost exactly equal to $E_a - RT$, where E_a is the Arrhenius activation energy.

TABLE I

RATE CONSTANTS, ENTHALPIES AND ENTROPIES OF ACTIVATION FOR HYDROLYSES OF SEVERAL PEPTIDES

Substrate	Medium	Relative rate (54.4°)	$k \times 10^6$ (liter mole^{-1} sec.$^{-1}$) 54.4°	70.2°	85.0°	ΔH^* (kcal.)	$-\Delta S^*$ (cal. deg.$^{-1}$)
Glycylglycine	HCl	1	1.13	4.96	17.2	20.3 ± 0.2	24.0 ± 0.5
			1.10	4.79	21.7[a]		
	Co^{++} added	1.7	1.86	6.93	24.6	18.9 ± 0.5	27.1 ± 1.4
	NaOH	47	51.4	183	608[a]	16.9 ± 0.1	26.8 ± 0.3
Acetylglycine	HCl	9.9	10.9	50.0	194	21.2 ± 0.2	16.6 ± 0.5
DL-Alanylglycine	HCl	0.56	0.624	2.58	10.5	20.5 ± 0.4	24.6 ± 1.0
			.615	2.43	9.72		
Glycyl-L-tyrosine	HCl	.52	.570	2.40	8.25	19.7 ± 0.1	27.1 ± 0.2
			.561	2.39	8.31		
Glycyl-L-leucine	HCl	.48	.531	2.32	7.39	19.8 ± 0.4	27.1 ± 1.0
			.529	2.01	8.24		
Glycyl-L-tryptophan	HCl	.44	.490	2.08	7.99	20.2 ± 0.5	25.8 ± 1.4
L-Leucylglycine	HCl	.18	.200	0.990	3.81	21.6 ± 0.1	23.4 ± 0.2
			.206	0.960	3.78		
			43.4°	54.4°	64.8°		
Glycylglycine	Dowex-50	16	6.39	18.2	52.5	20.1 ± 0.5	19.0 ± 1.4

[a] 87.2°.

of the resin, the rates are decreased slightly, but neither heat nor entropy of activation is significantly altered.

The hydrolysis of diglycylglycine in acid solution proceeds as

$$G_3^+ + H_3O^+ = G_2^+ + G^+ \qquad (k_3')$$
$$G_2^+ + H_3O^+ = 2G^+ \qquad (k_2')$$

The rate was followed by determination of free amino acid, G^+, by the ninhydrin method. In the presence of sufficient acid to maintain an effectively constant (H^+), an experimental condition satisfied during the initial stages of these runs, the rates will follow quasiunimolecular laws

$$-d(G_3^+)/dt = k_3'(H_3O^+)(G_3^+)$$
$$d(G_2^+)/dt = k_3'(H_3O^+)(G_3^+) - k_2'(H_3O^+)(G_2^+)$$
$$d(G^+)/dt = k_3'(H_3O^+)(G_3^+) + 2k_2'(H_3O^+)(G_2^+)$$

On substituting $k_3 = k_3'(H_3O^+)$, $k_2 = k_2'(H_3O^+)$, and setting $x = (G_3^+)$, $y = (G_2^+)$, $z = (G^+)$, and $a = (G_3^+)$ at $t = 0$, the integrated first-order equations are

$$x = ae^{-k_3 t}$$
$$y = \frac{k_3 a}{k_2 - k_3}(e^{-k_3 t} - e^{-k_2 t})$$
$$z = 3a - ae^{-k_3 t} + \frac{2ak_2k_3}{k_2 - k_3}\left[\frac{1}{k_2}e^{-k_2 t} - \frac{1}{k_3}e^{-k_3 t}\right]$$

Since k_2 is calculable from the work with glycylglycine, k_3 can be readily determined by the following procedure. Letting $\theta = k_3/k_2$, one obtains

$$(3 - z/a) = \frac{1}{\theta - 1}[2\theta e^{-k_2 t} + (\theta - 3)e^{-k_2\theta t}]$$

Both members of this equation are plotted as functions (f) of time in Fig. 4, the left member from the experimental data, and the right for various choices of the parameter θ until the best fit is obtained. In the later stages of reaction at the highest temperature there is a considerable deviation from the theoretical kinetics, owing perhaps to failure of the quasiunimolecular rate law. The constant k_3 is the sum of the constants for the breaking of the two different peptide links in the molecule.

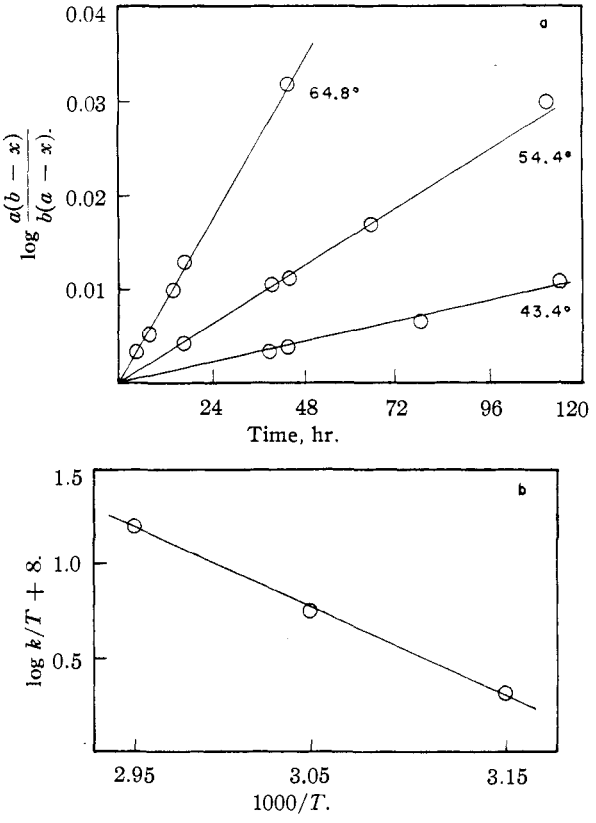

Fig. 3.—Hydrolysis of glycylglycine catalyzed by an ion-exchange resin, Dowex-50 (in the hydrogen-ion form): (a) data plotted according to a 2nd-order equation; (b) plot of log k/T *vs.* $1/T$.

The work of Smith[11] on the influence of heavy metals on peptidase activity suggested that it would be of interest to determine whether added metal ions would increase the rate of acid hydrolysis of glycylglycine. An equivalent concentration of cobaltous chloride was added to the solution of dipeptide in hydrochloric acid. The result was to de-

(11) E. J. Smith, *J. Biol. Chem.*, **163**, 15 (1946); *Proc. Nat. Acad. Sci.*, **35**, 80 (1949).

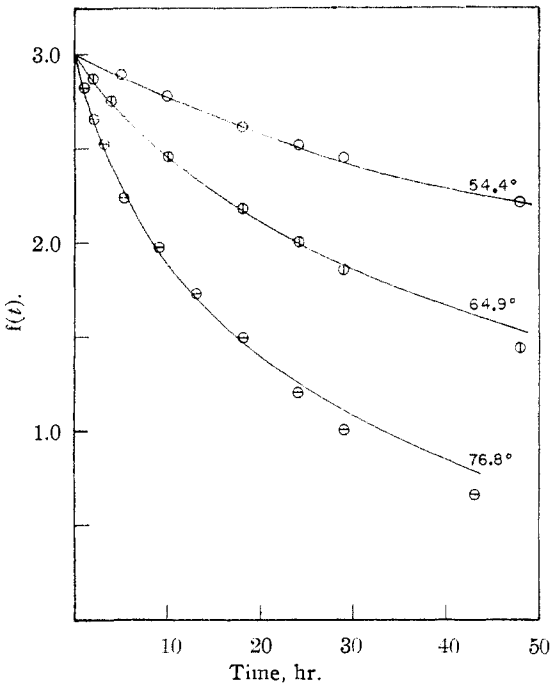

Fig. 4.—Hydrolysis of diglycylglycine. The circles are experimental; the curves, theoretical, for the parameter $\theta = 8$.

crease the activation energy of the hydrolysis by about 1.4 kcal. and to decrease the activation entropy by about 4 e.u. The identity and concentration of the cobalt complex of glycylglycine that may be formed are not known, but it is of interest that a definite effect of the heavy metal ion is observable in this simple system, and further work on the hydrolysis of well characterized peptide complexes should be instructive.

Basic Hydrolysis.—The experimental results on the basic hydrolysis yielded the rate constants ΔH^* and ΔS^* shown Table I.

Enzymatic Hydrolysis.—Only a preliminary study of the kinetics of carboxypeptidase-catalyzed hydrolysis has been completed, with substrate concentrations of 0.05 and 0.035 molar carbobenzoxyglycyl-L-phenylalanine, an enzyme concentration of about 10^{-8} molar (based on a molecular weight of 31,600), and at 15.0 and 25.0°. The concentration of active enzyme was calculated for each set of runs from an activity test by the method of Hofmann and Bergmann.[9] Over the small range of substrate concentration that was covered initial rates appeared to be first order with respect to substrate, S.

$$-\mathrm{d[S]}/\mathrm{d}t = k'[\mathrm{E}][\mathrm{S}]$$

Runs at 15 and 25°, plotted in accord with the first-order rate law, are shown in Fig. 5. The first-order constants at 15° show a mean deviation of 1.2% over 85% of the reaction; and at 25°, a mean deviation of 2.5% over 65% of the reaction.

Elkins-Kaufmann and Neurath[12] in a careful study of the kinetics at 25° have found K_m = 0.033. It would therefore appear that the present runs are at an initial substrate concentration somewhat higher than the optimum for good first-order constants.

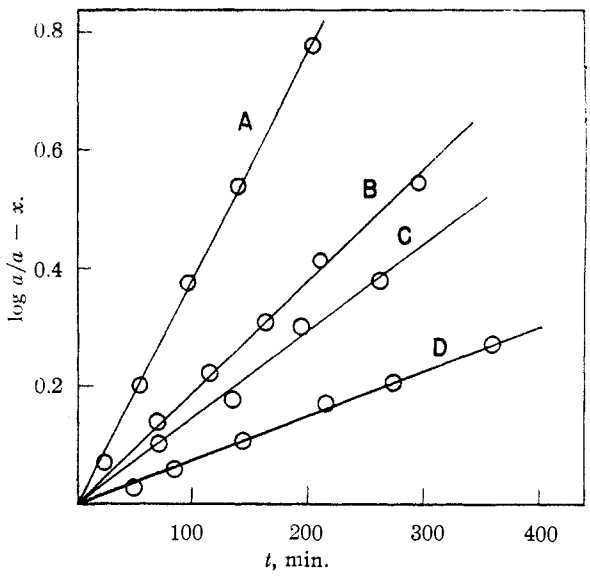

Fig. 5.—Hydrolysis of carbobenzoxyglycyl-L-phenylalanine by carboxypeptidase, (A) and (B): 25° and 15°; $S_0 = 0.0486\ M$; $E_0 = 3.90 \times 10^{-4}$ mg. N per cc.; (C) and (D), 25° and 15°; $S_0 = 0.0506\ M$; $E_0 = 1.15 \times 10^{-4}$ mg. N per cc. Data plotted according to first-order rate law.

The constants found are $k'[\mathrm{E}]^{-1} = 1.16 \times 10^3$ liters mole^{-1} sec.$^{-1}$ at 15°, and 2.27×10^3 liters mole^{-1} sec.$^{-1}$ at 25°. These correspond to $\Delta H^* = 10.9$ kcal. mole^{-1} and $\Delta S^* = -6.4$ cal. deg.$^{-1}$ mole^{-1}. Comparison of these figures with typical values for the acid or basic hydrolysis of peptides in Table I demonstrates that the catalytic enzyme lowers the activation energy and raises the activation entropy for peptide-bond hydrolysis.

Diglycylglycine.—The rate constants, k_3, for the hydrolysis of the first peptide bond in diglycylglycine, are about 8 times as high as the constants for the splitting of the dipeptide. Variation of $\theta(= k_3/k_2)$ gave the following values (if only 60% of the reaction at the highest temperature is considered): $\theta = 7.72 \pm 0.06$ at 54.4°, 8.01 ± 0.07 at 64.9°, and 7.80 ± 0.06 at 76.8°, yielding $\Delta H^* = 20.2$ kcal. and $\Delta S^* = -20.0$ e.u. for the constant k_3.

Discussion

Although the hydrolysis of peptides in the presence of strong acids or bases is commonly spoken of as a catalytic reaction, it does not follow the usual criterion of catalysis since one equivalent of oxonium ion or of hydroxide ion is consumed for each peptide bond that is broken.

Under comparable experimental conditions the acid hydrolysis of glycylglycine has a rate constant about $^1/_{10}$ that for acetylglycine and about 3 times that for benzoylglycine. The difference between the rates for acetylglycine and benzoylglycine is an entropy effect,[13] since the energies of activation for these substances appear to be about equal. Lower entropy accounts also for the reduction of the rate for glycylglycine relative to acetylglycine. The ac-

(12) E. Elkins-Kaufmann and H. Neurath, *J. Biol. Chem.*, **175**, 893 (1948).

(13) H. Eyring and A. E. Stearn, *Chem. Revs.*, **24**, 255 (1939), discuss this effect with reference to these two compounds.

tivation energy for the glycylglycine is in fact somewhat lower, presumably owing to the electrophilic action of the $-NH_3^+$ group, but the entropy effect is predominant.

The rates of hydrolysis of the peptides formed from glycine and leucine decrease in the order: glycylglycine, glycylleucine, leucylglycine. In leucylglycine, the substituent between the charged group and the peptide bond is electron-releasing, counteracting the charged group and thus strengthening the peptide bond relative to glycylglycine. Thus the lower rate is due to an energy effect. The lower rate of glycylleucine, on the other hand, is due to a marked entropy effect. As is often the case with aliphatic compounds, substituent effects are not large; from the data in Table I the total range in the free energies of activation for the dipeptides is from 28.9 to 29.6 kcal. mole^{-1}.

All these reactions have large negative entropies of activation, probably because of the loss of freedom when the oxonium ion is "hydrogen bonded" to the peptide cation in the activated complex. It may be noted that the "solid acid," Dowex 50, is a more efficient hydrolyzing agent than an ordinary acid, lowering the heat of activation by about 0.5 kcal. and raising the entropy of activation by 4.6 e.u. It would appear that the adsorption of the peptide at the acid group of the resin may be accompanied by the release of a water molecule from the resin, so that the decrease in entropy caused by the activated adsorption is somewhat less than in the ordinary acid hydrolysis. It is of interest that Haskell and Hammett[14] found that the resin acid is less efficient than hydrochloric acid in the hydrolysis of a series of alkyl esters in 70% acetone solution, the lower entropy of activation being more significant than the changes in the energy of activation. Thomas and Davies,[15] using the same resin (Amberlite IR-100) and the same choice of alkyl esters, report that in aqueous media the resin is more efficient than hydrochloric acid as a catalyst.

Since it appears that dipeptides are more resistant to hydrolysis than higher peptides[2,16] the hydrolysis of diglycylglycine under the same experimental conditions as those for glycylglycine was measured. The average of the two rate constants for the splitting of the first bond in the tripeptide is four times as large as that for the bond in the dipeptide, a change in the entropy of activation accounting pri marily for the change in rate. Kuhn[4] found that in sodium hydroxide solutions at 20° the ratio for the same two compounds was 2:1. Without further study, however, which bond in the tripeptide is split first cannot be definitely decided.

(14) V. C. Haskell and L. P. Hammett, THIS JOURNAL, **71**, 1284 (1949).

(15) G. G. Thomas and C. W. Davies, *Nature*, **159**, 372 (1947).

(16) A. H. Gordon, A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **34**, 1369 (1941); H. N. Christensen, *J. Biol. Chem.*, **154**, 427 (1944).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DUKE UNIVERSITY]

The Differential Soret Coefficient of Certain Electrolytes[1,2]

BY MANUEL GARCÍA MORÍN,[3] JOHN H. SAYLOR AND PAUL M. GROSS

A more rapid experimental method for investigating the Soret phenomenon is described. A distinction is made between the "integral" Soret coefficient which has been previously determined and the differential coefficient. The integral Soret coefficient of 0.100 *N* hydrochloric acid, sulfuric acid, lithium hydroxide, sodium hydroxide and potassium hydroxide is determined at different ranges of temperature which leads to the determination of the differential Soret coefficient of these substances at 25°, and of hydrochloric and sulfuric acids and sodium hydroxide at 35°. Using the "cage" model of the liquid state, the energy that opposes the departure of a "molecule" of 0.100 *N* hydrochloric acid from its cage at 25 and 35° is calculated as well as the energy necessary for the "molecule" to open a hole at the position of arrival. From these values, the heat of transport for HCl at 25 and 35° is evaluated.

While many physico-chemical quantities of value in the study of solutions are known with reasonable precision for a variety of solutions, for certain other quantities the available data are either of low precision or missing entirely. This is true of the Soret coefficient (or more properly the Ludwig–Soret coefficient).[4,5] A survey of the literature reveals wide discrepancies between the results obtained by different investigators, the existence of many gaps, and only limited data on the temperature dependence of this coefficient.

The present investigation points out the necessity of distinguishing between the "integral" Soret coefficients which have been previously determined and the more generally valid and useful differential coefficients. It also gives the preliminary results of the exploration of a more rapid and possibly a more generally applicable experimental method of investigating the Soret phenomenon.

In general, when a solution is subjected to a temperature gradient, a concentration gradient with opposite sign and direction appears in the solution. Each of these gradients gives rise to a flow of matter in opposite directions, so in time a steady state is attained characterized by zero net flow. If the temperature gradient is positive and has a vertical direction, the process is called the pure Soret effect. If the temperature gradient is applied in a horizontal direction, the thermogravitational effect of Clusius and Dickel[6] is obtained. The present paper deals with the determination of the Soret

(1) This paper is taken from a thesis submitted by Manuel García Morín to the Graduate School of Duke University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, June, 1948.

(2) Presented before the Division of Physical and Inorganic Chemistry at the 118th meeting of the American Chemical Society, Chicago, Illinois.

(3) Department of Chemistry, University of Puerto Rico, Rio Piedras, Puerto Rico. du Pont Predoctoral Fellow, Duke University, 1946-1948.

(4) C. Ludwig, *Sitzber Akad. Wiss. Wien*, **20**, 839 (1856).

(5) C. Soret, *Arch. sci. phys. et nat.* [3] **2**, 48 (1879); [3] **4**, 209 (1880), *Compt. rend.*, **91**, 289 (1880); *Ann. chim. phys.*, [5] **22**, 293 (1881).

(6) K. Clusius and G. Dickel, *Naturwissenschaften*, **26**, 546 (1938).