The Acid Catalysed Hydrolysis of Acetylglycine and Glycyltyrosine

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The hydrolysis of acetylglycine and glycyltyrosine have been carried out over a wide range of acidities in hydrochloric, perchloric, and sulphuric acids at 100·1 °C. Treatment of the rate data by Zucker–Hammett, Bunnett w and w^*, and Bunnett–Olsen linear free energy relationship criteria of mechanism indicates that hydrolysis takes place by a simple bimolecular A-2 mechanism.

SEVERAL workers [1] have undertaken kinetic investigations to determine the influence of electrostatic, polar, and steric factors on the rate of hydrolysis of dipeptides. Only one of these studies [2] covers the hydrolysis reaction over a range of acidities and the majority [3,4] refer to only one acid concentration.

The hydrolysis of acetylglycine has been studied in 0·1—8·2M-HCl at 61 °C,[2] in 2·0M-HCl in the temperature range 55—94 °C,[5] and in 0·6—0·8M-HCl between 54 and 85 °C.[4] That of glycyltyrosine has been studied in 0·6—0·8M-HCl at 54—85 °C [4] and in 2·0M-HCl at 99 °C.[3]

We report here data for hydrolysis of acetylglycine at 100·1 °C in 4·99—35·2 HCl, 5·34—80·0 H_2SO_4, and 5·08—60·0% w/w $HClO_4$ and for hydrolysis of glycyltyrosine at 100·1 °C in 4·99—30·4 HCl, 5·34—60·0 H_2SO_4, and 5·08—69·2% w/w $HClO_4$.

RESULTS AND DISCUSSION

Tables 1 and 2 give the pseudo-first-order rate constants of hydrolysis, k_ψ, in hydrochloric, sulphuric, and perchloric acids at 100·1 °C for acetylglycine and glycyltyrosine respectively.

TABLE 1

Hydrolysis of acetylglycine at 100·1 °C in hydrochloric, sulphuric, and perchloric acids

HCl (% w/w)	10^5k_ψ/s^{-1}	H_2SO_4 (% w/w)	10^5k_ψ/s^{-1}	$HClO_4$ (% w/w)	10^5k_ψ/s^{-1}
4·99	35·1	5·34	32·2	5·08	13·1
9·47	92·8	10·1	49·7	10·0	37·2
15·1	100	15·1	58·0	15·0	48·4
19·9	71·7	20·0	68·4	20·0	60·8
25·6	27·1	25·1	81·8	25·0	69·8
30·4	17·1	30·1	91·7	25·0	72·9
35·2	7·03	35·0	85·7	30·0	80·7
		40·0	60·7	35·0	61·3
		50·2	29·4	40·4	40·1
		60·0	12·3	50·2	17·9
		70·0	5·11	60·0	1·61
		80·0	0·282		

The rate profiles for hydrolysis of acetylglycine are bell-shaped (as previously found by Edward and Meacock [2]) and are similar to those generally found for amides.[6] However, the rate profiles for hydrolysis of glycyltyrosine are parabolic and are similar to those obtained for hydrolysis of sucrose [7] and benzonitrile [8] in these acids.

Acetylglycine is a moderately basic substrate ($pK_{BH^+} = -1.98$ [2]) and therefore the rate constants of

TABLE 2

Hydrolysis of glycyltyrosine at 100·1 °C in hydrochloric, sulphuric, and perchloric acids

HCl (% w/w)	10^5k_ψ/s^{-1}	H_2SO_4 (% w/w)	10^5k_ψ/s^{-1}	$HClO_4$ (% w/w)	10^5k_ψ/s^{-1}
4·99	1·49	5·34	4·64	5·08	1·17
10·0	9·13	10·1	5·95	10·0	2·34
15·1	26·4	15·1	8·72	15·0	3·98
20·0	46·2	20·0	15·1	15·0	3·91
25·6	76·2	25·1	19·4	20·0	5·33
30·4	250	30·1	30·8	20·0	5·40
		35·0	33·1	25·0	6·71
		40·0	38·2	30·0	9·41
		50·2	65·3	35·0	13·3
		60·0	102	40·4	20·2
				50·2	42·1
				60·0	76·5
				69·2	126

hydrolysis must be corrected for the degree of protonation of the substrate, $\alpha = h_A/(h_A + K_{BH^+})$, before any attempt at kinetic analysis is made. The H_A acidity function is used to calculate the proportion of ionised acetylglycine because this substrate is an amide. Although glycyltyrosine is a weakly basic substrate (no spectrophotometric evidence can be found for protonation over the range of acidities used for the rate measurements), it would seem reasonable to use the H_A function, rather than H_0, in any equations used to distinguish between mechanistic criteria. It must be noted, however, that Bunnett's original w values [9] and Bunnett and Olsen's ϕ values [10] were obtained using H_0 and Rochester [11] does not suggest the modification we have used below.

Values of H_A, the amide acidity function, in H_2SO_4,[12] HCl,[13] and $HClO_4$,[14] have been evaluated at 25 °C.

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[14] K. Yates, personal communication.

Values of H_0 in H_2SO_4 at 90 °C,[15] and in HCl [16] and $HClO_4$ [17] at 25 °C, and values of a_w calculated from vapour pressures of water at 60 and 100 °C in HCl and at 100 °C in H_2SO_4,[18] and from osmotic coefficients at 25 °C in $HClO_4$,[19] have been substituted into the calculations which follow. Substitution of parameters, measured at temperatures different from that of the rate data, into these calculations introduces some error but it is not regarded as significant.

Table 3, which summarises the analysis of the data for acetylglycine, gives the correlation coefficient of a plot of the Zucker–Hammett A-1 relationship for a moderate weak base, $\log_{10}k_\psi$ against H_0, the slope and correlation coefficient of the Zucker–Hammett A-2 relationship, $\log_{10}k_\psi$ against C_{H^+}, and Bunnett w relationship,[9] $\log_{10}k + H_A$ against $\log_{10}a_w$, the correlation coefficient of the Bunnett w^* relationship,[9] $\log_{10}k_\psi - \log_{10}C_{H^+}$ against $\log_{10}a_w$, and the slope, ϕ, the intercept, $-\log_{10}k_2^0$, and the correlation coefficient of the Bunnett–Olsen linear free energy relationship,[10] $\log_{10}k_\psi + H_A$ against $H_0 + \log_{10}C_{H^+}$, for hydrolysis of glycyltyrosine in sulphuric, perchloric, and hydrochloric acids at 100·1 °C.

Somewhat surprisingly, the least sophisticated criterion of mechanism, the Zucker–Hammett A-2 hypothesis, provides the best overall fit of the rate data. Bunnett w plots are curved but the values of w obtained are of the order expected for hydrolysis by an A-2 mechanism, and the values of ϕ are <0·58 and lie within the acceptable range for an A-2 mechanism.

Martin [20] proposed that the hydrolysis of glycylglycine in aqueous acetic acid occurred by a mechanism which was similar to that for hydrolysis of amides, *i.e.* an initial fast reversible addition of a proton to the peptide nitrogen was followed by the rate-determining attack of a water molecule on the carbonyl atom of the amide cation so formed.

In general, the rate constants for hydrolysis of glycyltyrosine and acetylglycine fit the mechanism suggested by Martin.

TABLE 3

Analysis of rate data for hydrolysis of acetylglycine by use of Zucker–Hammett, Bunnett w and w^*, and Bunnett–Olsen linear free energy relationships

Acid	Temp. (°C)	Z–H A-1 Correln. coefft.	Bunnett w w [a]	Bunnett w Correln. coefft.	Bunnett w^* Correln. coefft.	Bunnett–Olsen l.f.e.r. ϕ [b]	$-\log_{10}k_2^0$ [c]	Correln. coefft.
H_2SO_4	100·1	0·907	3·36	0·948	0·872	0·84	1·69	0·990
$HClO_4$	100·1	0·361	2·96	0·973	0·969	0·72	1·76	0·995
HCl	100·1	0·879	3·39	0·981	0·474	0·82	1·76	0·989
HCl [2]	61·0	0·983	3·60	0·977	0·844	0·81	3·05	0·991

[a] 0·37 > standard deviation(s) > 0·23. [b] 0·04 > s > 0·02. [c] 0·08 > s > 0·04.

TABLE 4

Analysis of rate data for hydrolysis of glycyltyrosine at 100·1 °C by use of Zucker–Hammett, Bunnett w and w^*, and Bunnett–Olsen linear free energy relationships

Acid	Z–H A-1 Correln. coefft.	Z–H A-2 Slope [a]	Z–H A-2 Correln. coefft.	Bunnett w w [b]	Bunnett w Correln. coefft.	Bunnett w^* Correln. coefft.	Bunnett–Olsen l.f.e.r. ϕ [c]	$-\log_{10}k_2^0$ [d]	Correln. coefft.
H_2SO_4	0·976	1·32	0·995	1·91	0·951	0·949	0·46	4·52	0·990
$HClO_4$	0·951	1·69	0·982	1·35	0·973	0·938	0·46	5·03	0·991
HCl	0·972	2·51	0·991	1·19	0·870	0·930	0·27	5·52	0·856

[a] 0·10 > s > 0·05. [b] 0·27 > s > 0·10. [c] 0·06 > s > 0·01. [d] 0·09 > s > 0·04.

Table 4 summarises the analysis of the data for glycyltyrosine and gives the correlation coefficient of a plot of the Zucker–Hammett A-1 relationship for a base,[11] $\log_{10}k_\psi - \log_{10}(1-\alpha)$ against H_0, the slope w and correlation coefficient of the Bunnett w relationship,[9] $\log_{10}k_\psi - \log_{10}\alpha$ against $\log_{10}a_w$, the correlation coefficient of the Bunnett w^* relationship,[9] $\log_{10}k_\psi - \log_{10}C_H/(h_0 + K_{BH^+})$ against $\log_{10}a_w$, and the slope, ϕ, the intercept, $-\log_{10}k_2^0$ (representing $\log_{10}k_2$ at infinite dilution in water), and the correlation coefficient of the Bunnett–Olsen linear free energy relationship,[10] $\log_{10}k_\psi - \log_{10}\alpha$ against $H_0 + \log_{10}C_{H^+}$, for hydrolysis of acetylglycine in sulphuric, perchloric, and hydrochloric acids at 100·1 °C. The data of Edward and Meacock in hydrochloric acid at 61 °C have also been analysed by the above criteria of mechanism. With the exception of the Bunnett–Olsen linear free energy relationship the correlation coefficients are poor. The ϕ parameters are all >0·58, and seemingly contradict the evidence provided by the values of w which are indicative of water being involved in the rate-determining step.

EXPERIMENTAL

Materials.—Acetylglycine (Koch–Light) was recrystallised from water, m.p. 207—208 °C. Glycyltyrosine (Sigma) was recrystallised from water.

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Apparatus.—An oil-bath was maintained at 100·1 ± 0·2 °C using a Gallenkamp contact thermometer, Klaxon stirrer, and a heating element. Spectrophotometric analyses were carried out on a Unicam SP 3000 spectrophotometer.

Hydrolysis Reactions.—Acetylglycine (10^{-3} g) and glycyltyrosine (0·7—1·2 × 10^{-3} g) were dissolved in the appropriate acid (10 ml), and aliquot samples were sealed in ampoules. These were removed at approximately 0, $\frac{1}{3}$, $\frac{2}{3}$, 1, $1\frac{1}{2}$, 2, and $2\frac{1}{2}$ half-lives and an infinite time sample was removed after heating for 8—10 half-lives. The hydrolysis was quenched by placing the sample in an ethanol–dry ice slurry.

The hydrolysis of acetylglycine was followed by observing the decrease in absorbance at 210 nm of aliquot samples diluted 1 in 10.

The hydrolysis of glycyltyrosine was followed by using the ninhydrin colorimetric method of Rosen [21] to analyse the amount of amino-acid present in the hydrolysed sample.

Least-squares analyses were carried out on an IBM 1130 computer.

[2/2070 *Received*, 1st *September*, 1972]

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