ACTA CHEMICA SCANDINAVICA 21 (1967) 2819–2822

Studies on Carbamates

XVI. The Carbamates of Glycylglycine and Glycylglycylglycine

OLE HENRIKSEN and MOGENS BALLUND JENSEN

The Royal Danish School of Pharmacy, Chemical Laboratory A, Copenhagen, Denmark

The velocity constants for the reaction between carbon dioxide and the amino group in glycylglycine and glycylglycylglycine have been determined. Equilibrium constants for the equilibria between carbamates and carbonates have been calculated. The velocities of decomposition of the carbamates in basic medium have been investigated and the experimental results interpreted in a way described in a previous paper.

The equilibrium conditions and the reaction mechanisms for the formation and decomposition of the carbamate formed by glycine in aqueous solution have been studied in a previous investigation in this series (Ref. 1).

The present investigation is concerned with the formation and decomposition of the carbamates of glycylglycine and glycylglycylglycine. The experimental methods (see below) were essentially the same as those used previously and the conditions for the formation and decomposition of the carbamates have been shown to be analogous to those of the carbamates investigated previously. Consequently, we shall only report the experimental data and the calculated constants obtained during these experiments, referring to previous investigations for further information (see, for instance, Ref. 2).

EXPERIMENTAL

Various preparations of glycylglycine were used in these experiments. These were provided by Nutritional Biochemical Corp., Dr. Theodor Schuchart, München, and Fluka. The equivalent weights, as determined by Kjeldahl's method for nitrogen determination, were 130.5, 131.7, and 131.3, respectively (theoretical value 132.1). Using the method of Fischer and Fourneau [3,4] we prepared glycylglycine hydrochloride monohydrate for use in some of the experiments. Using Kjeldahl's method, the equivalent weight was found to be 185.1 (theoretical value 186.6). The glycylglycylglycine was a Fluka product and the Kjeldahl analysis indicated an equivalent weight of 194.3 (theoretical value 189.2).

The method of analysis was similar to that used in previous investigations, but with some modifications which are given in a paper on the carbamates of some aminocaproic acids.[5] All of the experiments were carried out at 18°C and the velocity constants were calculated using the minute as the unit of time.

Aqueous solutions of glycylglycine and glycylglycylglycine are not stable for long periods of time due to hydrolysis of the peptide bonds; furthermore, this reaction is catalyzed by hydroxide ions. Therefore, the solutions were prepared immediately before use by adding carbon dioxide to amine-sodium hydroxide solutions. In the equilibrium experiments it was necessary to let the solutions stand for some days; however, these solutions are less alkaline than the amine-sodium hydroxide solutions. From the data on the velocity of hydrolysis given by Hammel and Glasstone [6] it can be estimated that the amount of hydrolysis is negligible in these experiments. Since the usual experiments on the velocity of decomposition of the carbamates in sodium hydroxide solutions could not be carried out, due to the above-mentioned hydrolysis of the peptide bonds, the decomposition of carbamate to carbonate was followed in a less basic medium, *viz.* a solution containing triethylamine and triethylammonium ion having a pH value of about 11. The reverse reaction — the conversion of carbonate to carbamate — is not negligible at this pH value, but it was suppressed by the addition of barium chloride to the reaction mixture which removed the carbonate as barium carbonate.

On the reaction "amine + carbon dioxide = carbamic acid"

The experiments on the absorption of carbon dioxide by solutions containing sodium hydroxide and glycylglycine or glycylglycylglycine are given in Table 1. The table also contains the calculated velocity constants for the addition of carbon dioxide to the amino groups, $k(CO_2 \cdot Am)$, and — for the sake of comparison — the value of $k(CO_2 \cdot Am)$ for glycine determined previously.[1] It is apparent that the addition of carbon dioxide to glycylglycine and glycylglycylglycine is about 3 times slower than the addition of carbon dioxide to glycine.

The equilibrium "carbamate = carbonate"

Table 2 gives the data from the equilibrium experiments. The carbamate solutions were prepared by shaking the amine-aminium ion solution with

Table 1. Carbon dioxide in peptides + NaOH. 18°C.

	Initial solution		Absorbed CO_2 moles/litre	% carbamate	Final solution		Mean		$k(CO_2 \cdot Am)$*	
	$[OH^-]$	[Am]			$[OH^-]$	[Am]	$[OH^-]$	[Am]		Mean
Glycine	Ref. 1									$10^{5.05}$
Glycylglycine	0.097	0.099	0.0122	35.2	0.077	0.094	0.087	0.097	$10^{4.63}$	$10^{4.63}$
	0.100	0.200	0.0195	47.4	0.070	0.191	0.085	0.195	$10^{4.61}$	
	0.110	0.290	0.0201	55.7	0.081	0.279	0.096	0.285	$10^{4.65}$	
Glycylglycyl-	0.094	0.098	0.0192	27.5	0.061	0.093	0.078	0.096	$10^{4.51}$	$10^{4.55}$
glycine	0.099	0.150	0.205	39.9	0.066	0.142	0.082	0.146	$10^{4.59}$	

* Briggs' logarithms.

Table 2. The solution of carbonate-carbamate in equilibrium. 18°C.

	Initial solution				% carbamate	Equilibrium				K_{Eq}	
	$[HCO_3^-]$	[carbamate]	$[AmH^+]$	[Am]		$[HCO_3^-]$	[carbamate]	$[AmH^+]$	[Am]		Mean
Glycine	Ref. 1										$10^{-1.48}$
Glycylglycine	0.022		0.040	0.060	75.5	0.0052	0.016	0.040	0.044	$10^{-1.85}$	$10^{-1.86}$
		0.021	0.041	0.038	77.7	0.0047	0.016	0.041	0.043	$10^{-1.91}$	
		0.015	0.055	0.029	73.3	0.0048	0.011	0.055	0.034	$10^{-1.81}$	
Glycylglycylglycine	0.0020		0.10	0.10	88.4	0.0022	0.017	0.10	0.082	$10^{-1.86}$	$10^{-2.01}$
		0.020	0.040	0.041	83.5	0.0033	0.016	0.040	0.044	$10^{-2.06}$	

carbon dioxide. Table 2 also gives the equilibrium constants, K_{Eq}, for the reactions

$$\text{carbamate ion} + H_2O = \text{Am} + HCO_3^-$$

$[HCO_3^-]$ has been set equal to the total concentration of carbonate in the calculations since the pH values of the amine-aminium ion solutions are close to the pH value of a solution of potassium bicarbonate in these experiments (pK_2 for glycylglycine is 8.17 and pK_2 for glycylglycylglycine is 7.91, both at 25°C [7]). Table 2 also contains the value of K_{Eq} for glycine determined previously.

The velocity of the conversion "carbamate= =carbonate"

The experimental data on the decompositions of the carbamates at pH approx. 11 are given in Table 3.

The experiments may be interpreted in the same manner as that used for the carbamates examined previously. The conversion is a two-step reaction:

carbamate = carbon dioxide
carbon dioxide = carbonate

and k_{amate} may be calculated from the expression

$$k_{\text{amate}} = \frac{k(CO_2\text{·Am}) \times K(H_2O) \times 1/K(CO_2) \times K_{Eq}}{[OH^-] + \dfrac{k(CO_2\text{·Am})}{k(CO_2\text{·OH})} \times [\text{Am}]}$$

The calculated values are listed in Table 3. Considering the nature of the experimental conditions, the agreement between calculated and experimental values is regarded as satisfactory.

Table 3. Velocity constants for the process: carbamate → carbonate. 18°C. pH = approx. 11.

	Initial solution		Min.	% carbamate left	k_{amate}
	[carbamate]	[Am]			
Glycylglycine	0.019	0.079	0	100	
			1146	45.3	0.000302
			1385	39.3	0.000294
			1514	37.7	0.000281
			2595	17.2	0.000295
			2974	13.0	0.000299
			3171	10.6	0.000307
					mean
					0.00030
					calc.
					0.00032
	0.021	0.18	0	100	
			1129	69.8	0.000138
			1477	61.5	0.000144
			2590	42.6	0.000143
			2769	39.6	0.000146
			4440	25.2	0.000134
			5632	14.2	0.000142
					mean
					0.00014
					calc.
					0.00015
Glycylglycylglycine	0.019	0.081	0	100	
			225	89.4	0.000220
			393	82.8	0.000208
			1323	50.3	0.000224
			1770	40.3	0.000222
			3260	17.3	0.000234
					mean
					0.00022
					calc.
					0.00023

Our thanks are due to Professor, Dr.phil. V. Sten Andersen, Head of Chemical Laboratory D, The Royal Danish School of Pharmacy, for his kind interest in this investigation.

REFERENCES

1. Jensen, A., Jensen, J. B. and Faurholt, C. *Acta Chem. Scand.* **6** (1952) 395.
2. Jensen, A. and Faurholt, C. *Acta Chem. Scand.* **6** (1952) 385.
3. Fischer, E. and Fourneau, C. *Ber.* **34** (1901) 2870.
4. Fischer, E. and Fourneau, C. *Ber.* **34** (1901) 436.
5. Jensen, M. B. and Faurholt, C. *Acta Chem. Scand.* **18** (1964) 377.
6. Hammel, E. F., Jr. and Glasstone, S. *J. Am. Chem. Soc.* **76** (1954) 3741.
7. Cohn, E. J. and Edsall, J. T. *Proteins, Amino Acids and Peptides*, Reinhold, New York 1942, p. 84.

Received July 5, 1967.