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The Thermal Polycondensation of Glycine in the Presence of Urea

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After the discovery of the polycondensation of glycine to form the glycine oligomer by heating in the presence of urea in a sealed tube, we investigated in order to establish the optimum reaction conditions as well as to study the reaction mechanism. The optimum conditions are attained when a mixture of 0.0133 mol (1 g) of glycine and 0.0016 mol (0.1 g) of urea is heated at about 160 °C for 20—30 hr. Under these conditions, an oligomer, composed of glycine units, with a polymerization degree of 17—18 is obtained with a 50—52% yield. The mechanism of oligomer formation is considered to be as follows: first, the urea is melted and part of it is used for converting the glycine to the corresponding amide. The glycinamide thus formed and the glycine dissolve locally in the molten urea, resulting in condensation between them. A repetition of the condensation leads to longer peptide chains.

The thermal polycondensation of glycine has long been investigated. Oligomers of glycine are formed by heating in the presence of glycerine,[1] the hippuric ester,[2] and mineral acids.[3] Recently, Orô and and Guidry[4] obtained the glycine oligomer with a polymerization degree of 12—18 by heating glycine in the presence of aqueous ammonia at 140—160 °C in a sealed tube, and Okamoto and Nakahara[5] reported that polyglycine with a high molecular weight was obtained by heating glycine in phosphoric acid.

The present authors also have investigated the thermal polycondensation of glycine for several years; so far they have obtained glycine oligomers with a polymerization degree of 10—20 by heating glycine in a sealed tube in the presence of water, inorganic acids, salts, and organic bases,[6] and organic acids.[7] We have also reported that the *N*-terminal of the oligomer thus obtained is acylated if organic acids are employed.

The authors chose urea as another possible effective polycondensation agent because it would not only act as a solvent, but would also be thermally decomposed into a biuret to evolve the NH_3 gas and would promote conversion to amide when heated with a carboxylic acid. They examined the thermal polycondensation of glycine in the presence of urea with a considerably high yield of the glycine oligomer. Then, some further examinations were made in order to establish the optimum reaction conditions and in order to clarify the reaction mechanism and the properties of the oligomer obtained. In this paper, the experimental data and results will be reported.

Experimental

Thermal Polycondensation. The sample, placed in a nitrogen-filled sealed tube (I.D., 1 cm; length, about 25 cm) was heated in a temperature-controlled oil bath at various temperatures for various periods of time. After the heating, the content of the tube was taken out and washed well with hot water. Then the residue was dialyzed for 24 hr in water which had been treated by an ion-exchanger and dried in a vacuum dessicator to obtain the glycine oligomer. Although the hot water contained a certain amount of unchanged glycine and a glycine oligomer with a polymerization degree of below 4, no attempt has been made to examine them.

Measurements. The average polymerization degree of the oligomer was determined by the Sörensen formol titration method (*C*-terminal determination) and the Van Slyke amino-nitrogen determination method (*N*-terminal determination) after the oligomer had been dissolved in 50% aqueous calcium chloride.

The IR spectra were measured by means of the Hitachi EPI-S2 infrared spectrophotometer.

Results and Discussion

Reaction Conditions and Properties of Oligomer. In order to examine the effect of the amount of added urea on the thermal polycondensation of glycine, a a given amount of urea (0—0.01 mol) was added to 0.0133 mol (1 g) of glycine, after which the mixture was heated at a constant temperature (130—170 °C) for 20 hr. Figure 1 shows the results. The optimum amount of urea depends upon the heating temperature. An excess of urea leads to lower yields of the oligomer. In our experiments, the highest yield of the oligomer was obtained when the reaction mixture was heated at about 160 °C in the presence of 0.0016 mol of urea. A pale yellow oligomer was obtained in a yield of about 51%. The relationship between the amount of urea and the polymerization degree of the oligomer is shown in Fig. 2. This shows that, as the amount of urea increases, the polymerization degree of the oligomer grows higher at the *C*- and *N*-terminal determinations, but the difference between them gradually

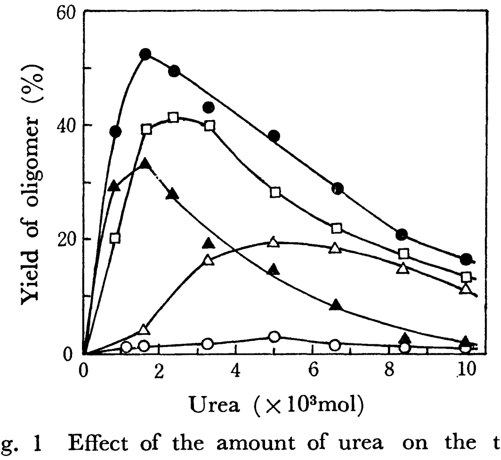

Fig. 1 Effect of the amount of urea on the thermal polycondensation of glycine.
Glycine: 0.0133 mol(1 g), Urea: 0-0.01 mol, Temperature: 130-170°C, Time: 20hr.
○: 130°C, △: 140°C, □: 150°C, ●: 160°C, ▲: 170°C

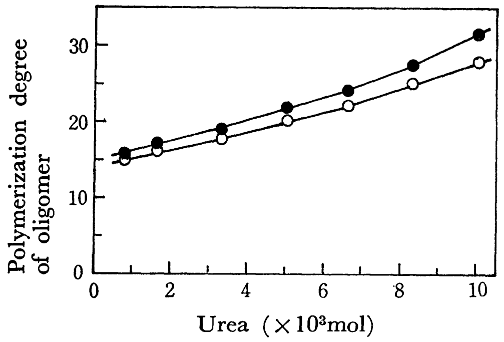

Fig. 2 Relation between polymerization degree of oligomer and the amount of urea on the thermal polycondensation of glycine.
Glycine: 0.0133 mol(1 g), Urea: 0-0.01 mol, Temperature: 160°C, Time: 20 hr.
○: *C*-terminal determination, ●: *N*-terminal determination

becomes greater and the polymerization degree in the latter case indicates a more or less higher value. This seems to be due to an irreversible decomposition of the glycine oligomer, judging from the facts that the reaction product in the sealed tube turned black or brown with urea concentrations higher than 0.005 mol, that the oligomer taken out of the tube was yellowish brown or brown, and that a reduction was observed in the yield of the oligomer.

Next, in order to examine the effects of the heating temperature and heating time, 0.0133 mol (1 g) of glycine and 0.0016 mol (0.1 g) of urea were mixed and heated to 140—180 °C for 0—100 hr. Figure 3 shows the results. According to the figure, the yield of the oligomer at each heating temperature increases with the heating temperature to reach a maximum. A higher temperature emphasizes this trend, but decreases the maximum yield of the oligomer. The relations between the heating temperature and the heating time for the yields higher than 50% are as follows:

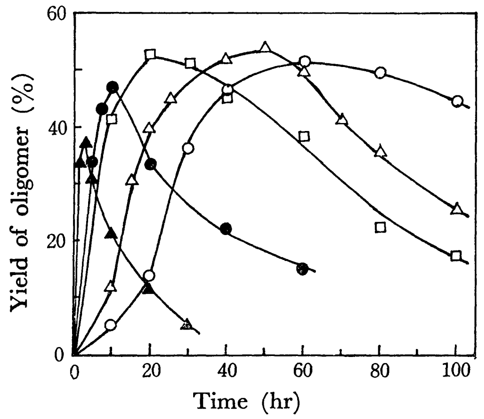

Fig. 3 Effects of heating temperature and heating time on the thermal polycondensation of glycine in the presence of urea.
Glycine: 0.0133 mol(1 g), Urea: 0.0016 mol(0.1 g).
○: 140°C, △:150°C, □: 160°C, ●: 170°C, ▲: 180°C

Temperatures	Heating times
140 °C	60—70 hr
150 °C	40—60 hr
160 °C	20—30 hr

The polymerization degree of the oligomer obtained under these conditions and that of the oligomers which provided the maximum yields at 170 °C and 180 °C were measured by means of the *C*- and *N*-terminal determinations. The results are tabulated in Table 1. This table shows that the polymerization degrees increase with the heating temperatures. When the heating temperature reaches 170 °C, however, a gradual deviation between the values obtained by the *C*- and *N*-terminal determinations becomes significant. As is shown in Fig. 4, a similar trend can be observed in the relations between the heating time and the polymerization degree of the oligomer at 160 °C. As elevated heating temperatures and extented heating times decreased the yields of the oligomer, as the mixtures turned dark, as the internal pressure of the sealed tube increased, and as CO_2 gas was detected in the NH_3–rich gas, an irreversible decomposition of the glycine oligomer might occur, as has been mentioned previously.

Table 1. Relation between heating temperature and polymerization degree of glycine oligomer
Glycine: 0.0133 mol (1 g), Urea: 0.0016 mol (0.1 g)

Heating temperature (°C)	Heating time (hr)	Polymerization degree of oligomer	
		C-terminal determination	*N*-terminal determination
140	60	11.0	12.0
150	50	13.2	13.8
160	20	16.5	17.0
170	10	18.1	20.2
180	3	19.4	22.5

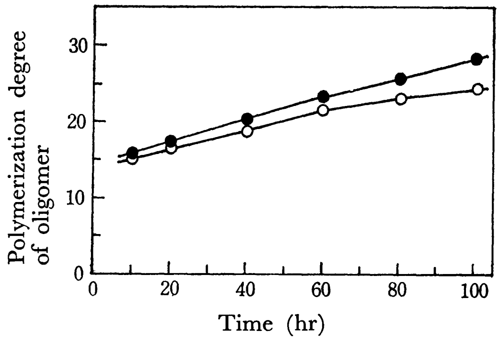

Fig. 4 Relation between polymerization degree of oligomer and heating time.
Glycine: 0.0133 mol (1 g), Urea: 0.0016 mol (0.1 g), Temperature: 160°C.
○: *C*-terminal determination, ●: *N*-terminal determination

From the experimental results so far obtained, it can be concluded that the optimum conditions for the polycondensation of glycine in the presence of urea occur when a mixture of 0.0133 mol (1 g) of glycine and 0.0016 mol (0.1 g) of urea is heated at 160 °C for

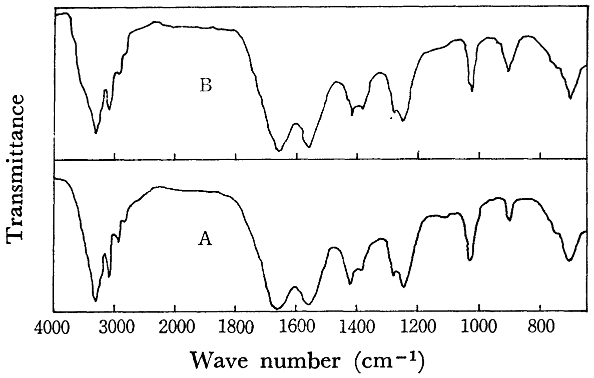

Fig. 5 Infrared absorption spectra (KBr).
A: Glycine oligomer obtained in the presence of urea,
B: Glycine oligomer obtained in the presence of water.

20—30 hr, with the polymerization degree of 17—18 and a yield of 50—52%. The oligomer thus formed is a pale yellow powder. It is soluble in concentrated aqueous solutions of calcium chloride, lithium bromide, or phosphoric acid, as well as in trichloroacetic acid, while it is very slightly soluble or insoluble in ordinary organic solvents and slightly soluble in an alkaline aqueous solution, presenting a distinct color change in a biuret reaction. The substance is influenced by heating in such a way that it begins to discolor at 230 °C, turns brown at 260 °C, and is completely carbonized at 280 °C. Furthermore, as is shown in Fig. 5, the IR spectrum of the oligomer found in the presence of urea coincides with that of the glycine oligomer obtained in the presence of water;[6] it showed typical peptide absorptions at 3300, 3070, 1648, and 1256 cm^{-1}, and a specific absorption at 1030 cm^{-1} based on the –Gly–Gly–connection. From the spectral analysis, it may be considered that the oligomer is composed of a single glycine unit.

Reaction Mechanism. As glycine undergoes polycondensation in the presence of urea with the evolution of CO_2, urea and glycine must first react with each other to form glycinamide. As is shown in Table 2, the polycondensation of glycine and glycinamide does not take place in the absence of glycerine, but the polycondensation of glycinamide alone does. However, a short heating treatment of the two substances in glycerine results in polycondensation between them, and through repeated condensation reactions, the peptide chains become longer with a considerably high yield of the glycine oligomer. Accordingly, urea may be considered to act as a solvent, just like glycerine, as well as converting glycine to glycinamide. Likewise, the use of a biuret instead of urea seems to give almost the same polycondensation results, as is shown in Table 3. Therefore, it may be considered that, as in the the case of urea, the biuret reacts with glycine to form glycinamide and acts as a solvent. Cyanuric acid, however, seems to show no such characteristics.

Table 2. Thermal polycondensation of glycine in the presence of glycinamide

Glycine: 0.0133 mol (1 g), Heating temperature: 160 °C

Reaction conditions			Oligomer		
				Polymerization degree	
Glycinamide (g)	Glycerine (g)	Time (hr)	Yield (g)	*C*-terminal determination	*N*-terminal determination
0.1	0	20	0.05	13.8	10.7
0.2	0	20	0.12	13.7	10.2
0.5	0	20	0.19	13.5	10.9
0.1	0.5	5	0.25	11.5	11.9
0.2	0.5	5	0.32	11.0	10.9
0.3	0.5	5	0.40	10.9	11.1
0	0.5	5	0.02	9.8	10.0

Table 3. Thermal polycondensation of glycine in the presence of biuret

Glycine: 0.0133 mol (1 g), Biuret: 0.97 mmol (0.1 g), Temperature: 160 °C

Heating time (hr)	Oligomer		
		Polymerization degree	
	Yield (%)	*C*-terminal determination	*N*-terminal determination
5	23.6	15.4	16.1
10	46.0	16.3	17.0
15	50.1	16.9	17.7
20	52.4	17.0	17.8
25	51.1	17.2	18.0
30	47.3	17.5	19.3
35	37.8	17.8	19.5

References

1) L. Balbiano and D. Trasiatti, *Ber.*, **33**, 1501 (1900); *ibid.*, **34**, 2323 (1901); L. C. Maillard, *Ann. Chim.*, **1**, 519 (1914); *ibid.*, **2**, 210 (1914).

2) T. Curtius and A. Benrath, *Ber.*, **37**, 1279 (1904).

3) A. B. Meggy, *J. Chem. Soc.*, **1953**, 851; *ibid.*, **1956**, 1444.

4) J. Orò and C. L. Guidry, *Arch. Biochem. Biophys.*, **93**, 166 (1961).

5) S. Okamoto and A. Nakahara, *Nippon Nogei Kagaku Kaishi*, **38**, 28 (1964).

6) H. Watanabe and Y. Kōzai, *Nippon Kagaku Zasshi*, **84**, 744 (1963).

7) H. Watanabe and Y. Kōzai, *ibid.*, **87**, 613 (1966); *ibid.*, **91**, 581 (1970).