STUDY OF THE EFFECT OF PRESSURE ON THE POLYCONDENSATION OF THE METHYL ESTER OF GLYCINE

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The present investigation forms a continuation of the work of Polyakova and Vereshchagin on the effect of pressure on the hydrolysis of 2,5-piperazinedione and the formation of polypeptide linkages [1]. The authors showed that increase of pressure greatly increases the rate of hydrolysis of 2,5-piperazinedione. Also, it was found that the final reaction mixture consisted not only of liquid hydrolyzate, but contained also a solid water-insoluble product. It was considered that the solid compound was a polypeptide formed from residues of hydrolyzed 2,5-piperazinedione.

Increase in pressure, therefore, not only leads to increase in the rate of hydrolysis of 2,5-piperazinedione, but also results in the formation of polypeptides from the amino acid molecules produced in the hydrolysis. The authors of this paper did not, however, succeed in determining the mean molecular weight of the solid compound, owing to its extremely low solubility.

The question of the effect of pressure on the rate of formation of polypeptides from amino acids and their esters is undoubtedly of great theoretical and practical interest. In view of this fact we have, in the present work, undertaken a study of the behavior of esters of amino acids under pressure. The reason for the choice of esters of amino acids as monomers is that the resulting alkoxyl end groups permit the number of units in the polymer chain formed to be determined comparatively readily, even when the polymer is insoluble in the usual organic solvents.

The simplest of the amino acid esters, the methyl ester of glycine, was selected for investigation. As early as 1888 Curtius [2], who was the first to obtain the methyl ester of glycine in the free state, found that, when this ester was left for a few days in a sealed tube, it was converted into a white solid mass. This phenomenon was given the name "polymerization", but its essential character was not discovered by Curtius. Later, in 1904 [3], the same worker showed that in the polymerization of the ethyl ester of glycine under like conditions a tetraglycyl compound was formed, namely \( \text{NH}_2\text{CH}_2\text{CO(NHCH}_2\text{CO)}_2\text{NHCH}_2\text{CO}_2\text{C}_2\text{H}_5 \), which was called a "bluret base" by the author. Curtius showed that, apart from the tetraglycyl compound, glycine anhydride was always present in the polymerization products. The next work on the polymerization of glycine esters appeared in 1939.

In a short communication Frankel and Katchalski [4] gave their experimental results on the polycondensation of the ethyl ester of glycine. The authors, who did not draw any theoretical conclusions, showed that by variation of the medium and temperature conditions, and by passage of gas through the ester, linear polymers of 12 to 20 units could be obtained. All of the polymers obtained were homolike in nature and were practically insoluble in hot water. The question of the polycondensation of glycine esters is treated in greater detail in a paper by the same authors, which appeared in 1942 [5]. The data supplied by this paper concerning the polycondensation of the methyl, ethyl, and isobutyl esters of glycine are mutually complementary. The authors showed that the methyl ester of glycine, when left in an open vessel for one month, yields a fraction that is insoluble in hot water and contains 18 amino acid residues. Also, when nitrogen is passed for 12 hours and the reaction mixture is set aside for one month at room temperature, a polymer of 30 units is obtained. When the methyl ester of glycine is kept in dry ether for three months at room temperature, a polymer of 27 units can be obtained, and when the ester is boiled in dry toluene for four hours and the reaction mixture is then left for three months, a polymer containing 35 monomer units is obtained.

Frankel and Katchalski consider that the glycine ester polymers that they isolated have a straight chain structure, this contention being proved, in their opinion, by the following facts, which they have established: 1) the proved presence of alkoxyl and amino end groups; 2) the obtaining of the bluret reaction during the alkaline hydrolysis of polymer molecules; 3) the quantitative agreement between the number of glycine residues in the condensation product (calculated from the content of alkoxyl groups) and the number of amino acid molecules liberated in the complete acid hydrolysis of the condensed molecules.

The paper of Fraenkel-Conrat et al. [6], which appeared in 1945 and was concerned with questions relating to the preparation of polyglycine esters, contains no data that are essentially different from those that we have cited. The authors kept the methyl ester of glycine in ether solution at room temperature for one month, and obtained, in addition to low polymers, highly polymeric glycine esters containing 15 glycine units. In confirmation of this value
of the chain length, the authors give analytical figures for free amino nitrogen, amino nitrogen liberated by acid hydroyis, and bound nitrogen.

It was considered to be of interest to study the polycondensation of the methyl glycine ester under super-high pressures at various temperatures. Also, it was considered to be important to establish the structure of the polymers with certainty, for the proof given by Katchalski of the linear-chain structure is not convincing: all of the arguments given above will apply also if there are cyclic groupings in the molecule, for example 2,5-piperazinedione units.

It must be noted that according to the 2,5-piperazinedione theory, which was advanced by Zelinsky in 1914 and developed by him, in conjunction with Sadikov in 1923 [7], the protein molecule, which is a polymer formed from amino acids, is bound to contain not only polypeptide chains, but also 2,5-piperazinedione rings. The researches of Zelinsky, Gavrilov, and coworkers have shown the readiness with which 2,5-piperazinediones are destroyed, and also the possibility of the formation of 2,5-piperazinedione rings under the conditions employed for the hydrolysis of proteins with the object of determining their structure.

Gavrilov and Koperina [8] have proposed a method of electrolytic reduction which, unlike previous methods for the determination of piperazinediones, permits the presence and number of piperazinedione rings in the protein molecule to be established with exactness. We have taken this method as our basis in proving the structure of polymers of glycine methyl ester formed under conditions of normal and super-high pressures.

In the present investigation we have studied the polycondensation of glycine methyl ether at 4500 atm and at various temperatures (room, 50, 75, and 130°). At the same time, comparative experiments were carried out at atmospheric pressure. As a result of the reaction a mixture of polycondensation products was obtained, from which we separated only the macromolecular polymers, insoluble in hot water. Investigation of the reaction products showed that high pressure results in increased rate of reaction and in the degree of polycondensation. In order to decide the question of the structure of the polymers obtained, we subjected them to electrolytic reduction by Gavrilov and Koperina's method.

Our tests showed that our polymers would not undergo the electrolytic reduction reaction that was to be expected if piperazinedione rings were present in the polymer molecule. This gave us reason to suppose that linear-chain molecules may be formed when polycondensation occurs under high-pressure conditions. Calculations of the theoretical contents of amino nitrogen and methoxyl groups based on a linear-chain structure for the polycondensation products were in good agreement with the experimental results. Since the polymers obtained were insoluble in all of the organic solvents that we tried, we could determine the molecular weight (and so the number of monomer units in the polymer) only by the chemical method: by the content of methoxyl end groups. We determined the methoxyl content by Vieböck's method, and the analytical results were confirmed by hydrolysis followed by determination of amino nitrogen by titration according to the methods of Sorensen and Lindström-Lang, and also by the preparation of copper salts.

**EXPERIMENTAL**

**Preparation of Starting Materials.** The methyl ester of glycine was prepared by the method proposed by Frankel and Katchalski in which the hydrochloride of glycine methyl ester is suspended in absolute ether and decomposed by a current of dry ammonia at 0°; it had the following constants: b.p. 46.5°(12 mm); 48.5°(16 mm); 54° (20 mm). For glycine methyl ester the literature gives: b.p. 45°(20 mm) [5]; b.p. 130°(760 mm) [6]; 54°(50 mm), average yield 48.4% [2]. In its turn the hydrochloride of glycine methyl ester was prepared by the following series of reactions: glycine $\xrightarrow{\text{HCl}}$ glycine hydrochloride $\xrightarrow{\text{HCl, C}_2\text{H}_5\text{OH}}$ hydrochloride of glycine methyl ester, m.p. 174.5-178° (the literature [2] gives 175°).

**Found %:** C 28.73; 28.94; H 6.62; 6.59; N 11.44; 11.32

**C$_2$H$_4$O$_2$NCl. Calculated %:** C 28.69 H 6.42 N 11.16

**Experimental Procedure.** The experiments on polycondensation under pressure were carried out in a strong tube of plastic material, length 10 cm, internal diameter 8.5 mm, wall thickness 2.5 mm. The ends of the tube were provided with pistons, by means of which the pressure was communicated to the substance under investigation; the tube was encased in copper. It was placed in a high-pressure reactor filled with freshly distilled, anhydrous glycerol. The reactor was immersed in an oil bath provided with electric heater, stirrer, and contact and control thermometers. The pressure was applied by means of a hydraulic compressor. The variation from the stipulated experimental temperatures did not exceed ± 1°.

The routine was the same for all of the pressure experiments; it was as follows: For the first 18 hours the glycine methyl ester was under pressure at room temperature. Heat was then applied for six hours (pressure experiments