KINETICS AND CHEMISTRY OF THE POLYCONDENSATION
ESTERS OF $\alpha$-AMINO ACIDS

COMMUNICATION 4. COPOLYCONDENSATION OF GLYCINE ETHYL ESTER AND
N-CARBOXYGLYCINE ANHYDRIDE

K.T. Poroshin, T.D. Kozarenko and Yu.I. Khurgin

The condensation of anhydrides of $\alpha$-(carboxyamino) acids with esters of amino acids was applied by Wessely and Sigmund [1] and Bailey [2] in the preparation of di- and tri-peptides. The lowness of the yield of peptides of the required structure and the formation of by-products (piperazinediones and higher peptides) considerably limited the extension of this method.

In our investigation we have carried out the copolycondensation of glycine ethyl ester and N-carboxyglycine anhydride, in which we expected that a process of mutual initiation would occur. The primary process (1) is initiation of the breakdown of the anhydride of the $\alpha$-(carboxyamino) acid by the amino group of the corresponding amino acid. The formation of a peptide chain by reaction of the anhydride of the carboxyamino acid is accompanied by liberation of carbon dioxide, which acts as an initiator for the polycondensation of the ester of the amino acid (2). Process 2 is accompanied by liberation of alcohol, which forms a second initiator for the polycondensation of the anhydride of N-carboxyglycine (3). Copolymerization is characterized by increase in the concentration of initiators and can therefore be regarded as an autocatalytic process. The scheme of mutual initiation can be represented as follows:

$$ \begin{align*}
R-CH-CO\rightarrow & -\text{NHCHRCO} - \\
NH-CO\rightarrow & -\text{COO} - \\
H_2N\text{CHR-COO}R' & \rightarrow -\text{NHCHRCO} - \\
& \text{R'}\text{OH}
\end{align*} $$

We investigated the chemical compositions of copolycondensation products obtained under identical conditions of temperature ($40^\circ$ for four hours) with different relative concentrations of glycine ethyl ester and N-carboxyglycine anhydride, which will be expressed as ratios of molar concentrations of anhydride and ester (A/E). Chromatographic investigation of the copolycondensation products showed that peptide esters were formed in the course of the reaction, but no free peptides were detected, which suggests that the peptide chain grows at the amino end.

EXPERIMENTAL

The copolycondensation of glycine ethyl ester and N-carboxyglycine anhydride was carried out both in the mass — by dissolving the appropriate proportion of one monomer in the other — and also in dry dioxane solution. For reaction in the mass the reaction vessel was kept in a thermostat at $40 \pm 0.1^\circ$ for four hours, and for copolycondensation in solution reaction was for 12 hours at the same temperature.

Mass copolycondensation was carried out for values of A/E of 0.02, 0.04, 0.067, 0.08, 0.168 and 0.233, and
Chromatographic separation of products of the copolycondensation of glycine ethyl ester and N-carboxyglycine anhydride in the system butyl alcohol + acetic acid + water (4:1:5) after threefold development: 1) mixture of glycine and the di-, tri-, tetra-, penta- and hexapeptides of the glycine series; 2) product of the copolycondensation of glycine ethyl ester and N-carboxyglycine anhydride (A/E 0.08; 40°; 4 hours); 3) product of the copolycondensation of glycine ethyl ester in presence of CO₂ [3] (CO₂/E 0.08; 40°; 4 hours).

For solution copolymerization the ratios were 0.1, 0.25, 0.45, 0.66, 1.0, 1.5, 2.0, 3.0 and 9.0.

At the end of reaction in the mass, the reaction mixture was cooled, and the solid part was ground with dry ether, filtered off, and again washed with ether.

The reaction products from the solution-copolymerization reaction were freed from dioxane at 40° under low pressure and then treated as in the first case.

The combined filtrate was analyzed for the content of unchanged glycine ethyl ester by the method described previously [3]. The same filtrate was tested for N-carboxyglycine anhydride by the method of Berger, Sela and Katchalski [4]. Chromatographic investigation of the ethereal filtrate showed that it contained glycine ethyl ester and traces of glycyglycine ethyl ester. In no case was N-carboxyglycine anhydride detected. The polymer, which contained peptide ethers of various chain lengths, was investigated by the method of differential titration developed previously [5] for the contents of triglycine esters, the "tetrapeptide" fraction, and 2,5-piperazinedione [6]. The mass-copolycondensation products were investigated by partition chromatography on paper. The chromatograms were formed in the systems butyl alcohol + acetic acid + water (4:1:5) and phenol + water (4:1) on chromatographic paper supplied by the Leningrad factory Goznak.

In the first system the peptide esters can be readily separated. Free peptides and glycine have low RF values and are not separated in this system. No peptides or glycine were detected in the condensation products. A chromatogram was taken also by Keil's method [7], and in this also no free peptides were detected. The chromatograms were color-developed by Rydon and Smith's method [8], the chlorinated chromatograms being treated in a solution of o-tolidine and KI [9] (see figure).

Investigation of the polymers obtained showed that they did not contain N-carboxyglycine anhydride. The content of tripeptide esters varied from 10.2% to 33.6%. The content of tetrapeptide and higher peptide esters varied from 20.10% to 48.35%. Piperazinediones were present in the polymers in amounts depending on A/E and varying from 16.10% down to scarcely detectable amounts.

**SUMMARY**

1. Copolycondensation of N-carboxyglycine anhydride and glycine ethyl ester was carried out in the mass and in solution for various relative amounts of monomers. The chemical compositions of the products were studied.

2. Copolycondensation proceeds under conditions of mutual initiation. No N-carboxyglycine or free peptides were detected in the copolycondensation products, but peptide esters and various amounts of piperazinediones were present.

3. It is suggested that the peptide chain grows from the amino end.