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Polymerization of Glycine Using Anilinium Triphosphate

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The polymerization of glycine was carried out in the presence of anilinium triphosphate at 150 °C under nitrogen. The conversion reached 81% after 15 h. The polyglycine obtained was slightly soluble in water and the mean molecular weight of the polymer was 800. Several neutral amino acids also gave conversions of 33 to 71%.

Keywords—condensation; polymerization; glycine; polyglycine; anilinium triphosphate

Polyamino acids, particularly oligopeptides, are potentially useful as surfactants, skin-protective drugs, additives, *etc.*, because of their amphoteric properties and good affinity for human skin. In the polymerization of amino acids, phosphoric acid or high-energy phosphates are known to form peptides catalytically. Fox and Harada¹⁾ have obtained a polypeptide, "proteinoid," by heating a mixture of 20 kinds of amino acids in pyroglutamic acid in the presence of phosphoric acid. As a model reaction for the origin of protein on the primitive earth, Sawai and Orgel²⁾ have identified several oligoglycines formed by heating glycine with adenosine triphosphate on a glass-fiber plate. Further, various acids and bases have been used for the polymerization of glycine in a closed system at elevated temperatures.^{3,4)}

We prepared anilinium triphosphate (AnTPP) and carried out the polymerization of glycine conveniently in an open system. Glycine (15 g, 0.2 mol) and AnTPP (7.2 g, 0.011 mol) were mixed and heated under a nitrogen flow at 150 °C. The amino acid and AnTPP melted during half an hour, and dehydration took place vigorously. The results of the condensation of glycine in the presence of AnTPP or anilinium pyrophosphate are shown in Fig. 1. The viscous liquid phase became turbid after 3 h and then solidified gradually. The conversion reached 81.3% after 15 h. By washing the product with water, a white to gray powder was obtained in a good yield (10 g). The polymer yields and the degrees of polymerization

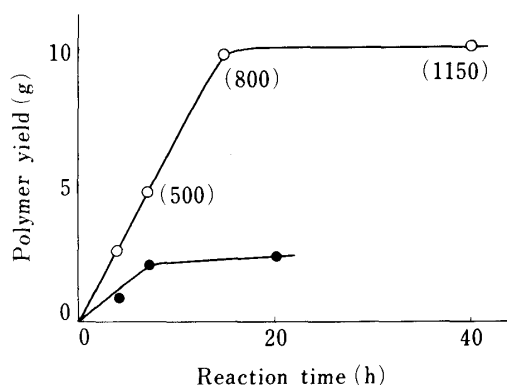


Fig. 1. Reaction Time-Yield Relationship in the Polymerization of Glycine

○, glycine 15 g and AnTPP 7.2 g; ●, glycine 15 g and AnPP 5.5 g. Reaction temp., 150 °C. (), mean molecular weight.

increased with increasing reaction time, and a molecular weight of 800 was attained. The degrees of polymerization were determined by the 2,4-dinitrophenylation of N-terminal glycine followed by the hydrolysis.⁵⁾ Prolonged heating or an elevated temperature gave a carbonaceous by-product. The glycine polymer was slightly soluble in water and easily soluble in formic acid. The formation of the peptides was confirmed by both the biuret response and the infrared (IR) absorptions at 1560 and 1656 cm^{-1} . However, the polymerization of glycine by using such polyphosphates as tetrasodium pyrophosphate and pentasodium tripolyphosphate failed (these salts have no melting points). The activity of orthophosphoric acid (85%) was poor; a 43.6% conversion and a polyglycine yield of 3.4 g were obtained from glycine (15.0 g) and 85% H_3PO_4 (3.0 g) at 150 °C for 15 h. Further heating did not improve the conversion; the reaction system solidified and the liberation of water ceased. In the case of tetraanilinium pyrophosphate, the reaction mixture remained in the solid state from the beginning of heating. In this case, the melting point of anilinium pyrophosphate (158–160 °C) exceeds the reaction temperature (150 °C). Therefore, a fusible salt of polyphosphoric acid was essential for the polymerization of glycine under our reaction conditions.

In the proton nuclear magnetic resonance (^1H -NMR) spectrum (DCOOD) of the polyglycine, an aromatic proton signal (δ 7.30) and a methylene proton signal (δ 4.30) were observed. The results suggest that the terminal carboxyl groups of the polyglycine have been modified with aniline of AnTPP to give polyglycine anilide. The ratio of the phenyl protons to the methylene protons was 1:4.8. The ratio corresponds to a molecular weight of 777 ($\text{C}_6\text{H}_5\text{NH}(\text{COCH}_2\text{NH})_n\text{COCH}_2\text{NH}_2$, $n=11$), which is in good agreement with the mean molecular weight of 800 obtained by the dinitrophenylation method. The elemental analysis of the hygroscopic polymer gave reasonable values for the hydrate of polyglycine anilide.

The polymerization of several kinds of neutral amino acids (1 g) was also carried out in the presence of AnTPP (1 g) at 150 °C for 6 h. It took 0.5 to 2 h to obtain a homogeneous melt with such amino acids as L-alanine, L-valine, L-leucine, L-isoleucine, and L-phenylalanine. The conversions were 70.6, 29.8, 50.2, 33.2, and 65.3%, respectively. The products were all positive in the biuret reaction.

Experimental

The ^1H -NMR spectra were recorded on a Hitachi R-600 high-resolution NMR spectrometer with tetramethylsilane (TMS) as an internal standard. The unreacted amino acids were analyzed with a Hitachi KLA-5 amino acid analyzer. The IR spectra were taken on a Hitachi 270-30 infrared spectrophotometer. Glycine and other amino acids of guaranteed reagent grade were purchased from Wako Pure Chemicals Ind., Ltd., Osaka, Japan. Phosphoric acid, tetrasodium pyrophosphate, and pentasodium tripolyphosphate were obtained from Rinkagaku Kogyo Co., Ltd., Shinminato, Japan. Melting points were obtained by using a Yanagimoto micro melting point apparatus (values are uncorrected).

Anilinium Pyrophosphate and Tripolyphosphate—The phosphates were prepared according to the method of Tanaka *et al.*⁶⁾ Tetrasodium pyrophosphate (13.3 g, 0.05 mol) was dissolved in water (100 ml). The solution was acidified with aqueous 35% HCl (21.0 g, 0.2 mol) under cooling, and aniline (18.6 g, 0.2 mol) was added dropwise under vigorous stirring. A white solid was precipitated. Yield, 15.0 g (54.5%). The precipitate obtained was recrystallized from water. Colorless plates, mp 158–160 °C. *Anal.* Calcd for $\text{C}_{24}\text{H}_{32}\text{N}_4\text{O}_7\text{P}_2 \cdot \text{H}_2\text{O}$: C, 50.70; H, 6.03; N, 9.86. Found: C, 50.05; H, 5.79; N, 9.66. Aqueous 35% HCl (104.3 g, 1.0 mol) was added to an aqueous solution of pentasodium tripolyphosphate (73.6 g, 0.2 mol) in 500 ml of water under cooling. Aniline (93.0 g, 0.1 mol) was added dropwise to the mixture under stirring. A white solid that precipitated was dissolved by heating at 60 °C and the solution was filtered. Colorless plates were obtained by cooling the solution, and recrystallized from water. The product was identical with tetraanilinium tripolyphosphate; mp 136–137 °C. Yield, 52.0 g (41.3%). *Anal.* Calcd for $\text{C}_{24}\text{H}_{33}\text{N}_4\text{O}_{10}\text{P}_3$: C, 45.72; H, 5.28; N, 8.89. Found: C, 45.70; H, 5.53; N, 8.34.

Polyglycine—The reaction products were dissolved in water and neutralized with aqueous NaOH. Fine precipitates were collected by centrifugation (3000 rpm), washed three times with water, and dried at 80 °C. A white to gray powder was obtained as the hydrate of polyglycine anilide, $\text{C}_6\text{H}_5\text{NH}(\text{COCH}_2\text{NH})_{11}\text{COCH}_2\text{NH}_2 \cdot 3\text{H}_2\text{O}$. *Anal.* Calcd for $\text{C}_{30}\text{H}_{43}\text{N}_{13}\text{O}_{12} \cdot 3\text{H}_2\text{O}$ (MW 831.79): C, 43.32; H, 5.94; N, 21.89. Found: C, 43.36; H, 5.56; N, 21.11.

Mean Molecular Weight of Polyglycine—The peptide (2 mg) was treated with 2,4-dinitrofluorobenzene

(DNFB) (0.1 g), aqueous 1% trimethylamine (1 ml), and L-threonine (1 μ mol) as an internal standard. The mixture was stirred for 2 h at room temperature. After the reaction, unreacted DNFB was extracted three times with ethyl ether (2 ml) and once with ethyl acetate (2 ml). The aqueous layer was evaporated on a water bath and the residue was sealed with 5 N HCl (2 ml) in a glass ampule under an N₂ atmosphere. The ampule was heated at 105 °C for 15 h. After the hydrolysis, the dinitrophenylated (DNP) amino acids were extracted with ethyl acetate and were developed by two-dimensional paper chromatography using *n*-butyl alcohol-H₂O-NH₃ followed by a 1.5 M phosphate buffer solution. The DNP amino acids extracted with aqueous 1% Na₂CO₃ were analyzed colorimetrically at 360 nm. The mean molecular weight (MW) was calculated according to the following equation

$$\overline{\text{MW}} = \frac{\text{sample (g)}}{\text{DNP amino acid (mol)}}.$$

References and Notes

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