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Temperature Effect on the Molecular Weight and the Optical Purity of Anhydropolyaspartic Acid Prepared by Thermal Polycondensation

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Synopsis. Anhydroyplyaspartic acid (APAA) was prepared by the thermal polycondensation of L-aspartic acid in the solid state at 160—220 °C for 5 h. The effect of temperature on molecular weight and optical purity of APAA was investigated. The molecular weight increases rapidly in the temperature range 180—200 °C, the yield of APAA also increasing rapidly. The optical purity decreases rapidly with rise in temperature from 160—180 °C.

In recent years, the thermal polycondensation of α-amino acids has drawn attention in view of the prebiotic formation of polypeptides. Aspartic acid yields anhydropolyaspartic acid (APAA) by thermal polycondensation in the solid state at around 200 °C.[1] APAA was converted into polyaspartic acid (PAA) having α- and β-aspartyl residues by alkaline hydrolysis.[2] Studies on the chemical structure[2-5] and potentiometric titration behavior[6] for APAA and PAA have been reported. However, little attention has been paid to the temperature effect on the physical properties of these polyamino acids prepared by thermal polycondensation.

In the present study, the effect of reaction temperature on the molecular weight, yield and optical purity of APAA was examined.

Experimental

Materials. L-Aspartic acid (10.0 g, 0.0752 mol) was packed tightly on the bottom of a 0.5 litre Erlenmeyer flask and was heated at various temperatures (160—220 °C) for 5 h under nitrogen atmosphere. The reaction time (5 h) was chosen since the yields of APAA become almost constant by heating at 180 and 200 °C for 5 h.[7] The slightly colored product was suspended in water and then dialyzed against distilled water for 5—6 days.[3] The weight losses of the products obtained at 160 and 220 °C during the dialysis are 97 and 29%, respectively. The insoluble material was collected by filtration and washed with distilled water and ethanol. APAA obtained was dried at 120 °C over phosphorus pentaoxide under reduced pressure for 4 days. PAA was prepared from APAA in the same way as described earlier.[6,7] APAA and PAA were characterized by infrared spectra and potentiometric titrations. PAA was titrated with 0.1 M NaOH, whereas APAA previously treated with 0.1 M NaOH was back-titrated with 0.1 M HCl.

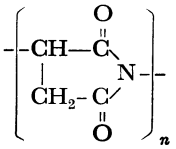
Viscometric Measurement. Viscometric measurements were carried out to investigate the change of molecular weight of APAA by reaction temperature. APAA was dissolved in 0.5 M NaOH and allowed to stand at 25 °C for 24 h. Measurements were carried out at 25±0.01 °C with a Ubbelohde capillary viscometer with 388.5 s flowing time for water at 25 °C. The reduced viscosity (η_{sp}/C) was determined at a constant concentration (C=0.179 g/dl).

Titration of N-Terminal Amino Groups with Perchloric Acid. The molecular weight of PAA derived from APAA prepared at various temperatures was determined by means of N-terminal amino group titration. PAA (20 mg) was dissolved in 5 ml of purified *N,N*-dimethylformamide. The sample solutions were titrated with 0.02 M perchloric acid with a microburet. The crystal violet was used as an indicator.

Determination of Optical Purity. APAA (20 mg) was hydrolyzed with 6 M HCl (5 ml) in a sealed tube at 110 °C for 24 h under reduced pressure. The hydrolyzate was evaporated to dryness, and the residue was dissolved in 6 M HCl. The molar ellipticity [θ] was measured with a JASCO model J-20C recording spectropolarimeter at wavelength 236 nm. On the other hand, the [θ] value at 236 nm for a standard solution of L-aspartic acid in 6 M HCl was also obtained. The concentrations of aspartic acid in the hydrolyzed samples and the standard solution (0.05—0.1 mol/l) were measured with a Yanagimoto model LC-5S amino acid analyzer. The optical purity of anhydroaspartyl residue in APAA was estimated by the ratio of the [θ] value of the sample solution to that of the standard solution.

Results and Discussion

APAA prepared by thermal polycondensation is mainly composed of the imide structure and is quantitatively hydrolyzed to aspartic acid.[2,3,7] The



structure is also supported by the results of potentiometric titration and infrared spectra. The molecular weight of repeating unit was estimated to be 99±2 for APAA and 115±1 for PAA by means of potentiometric titration. The infrared spectrum of APAA shows moderate absorption at 1780 cm^{-1}

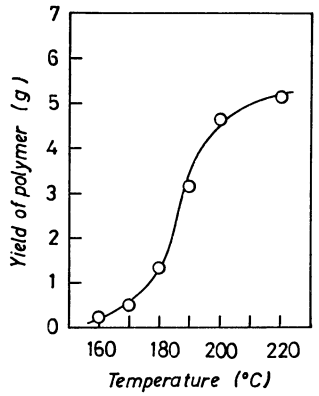


Fig. 1. Temperature effect on the yield of APAA. The yield is represented by the weight of APAA obtained from 10.0 g of L-aspartic acid.

and strong absorption at 1715 cm^{-1}, indicating the presence of 5-membered cyclic imide,[3,8] and moderate absorption at 1530 cm^{-1}(amide II). The infrared spectrum of PAA shows strong absorption at 1720 cm^{-1} (COOH), 1650 cm^{-1} (amide I), and 1530 cm^{-1} (amide II).

The relationship between the yield of APAA and the reaction temperature is shown in Fig. 1. It is seen that the yield of APAA rapidly increases with rise in reaction temperature from 180—200 °C. On the other hand, aspartic acid hardly undergoes polycondensation at all by heating below 160 °C.

The effect of reaction temperature on the molecular weight of APAA was investigated by means of viscometric measurement for APAA in 0.5M NaOH, and N-terminal amino group titration for PAA prepared from APAA. The results are shown in Fig. 2. Assuming that degradation of the polypeptide chain does not occur during the course of partial alkaline hydrolysis of the imide structure of APAA, we can estimate the molecular weight of APAA by means of the N-terminal amino group titration of PAA. The result obtained indicates that the molecular weight of APAA increases rapidly in the temperature range 180—200 °C. This is also supported by the result obtained by the viscometric measurement of APAA. It is evident that the molecular weight of APAA increases rapidly in the temperature range at which the yield of APAA increases rapidly.

The effect of reaction temperature on the optical

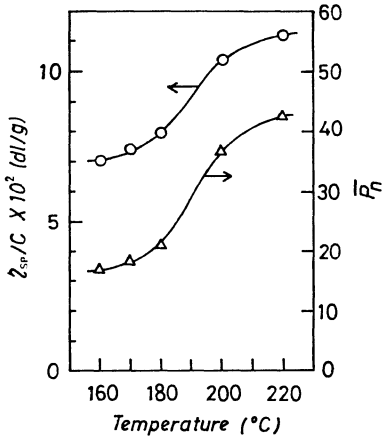


Fig. 2. Temperature effect on the molecular weight of APAA.
The reduced viscosity (η_{sp}/C) of APAA was determined in 0.5 M NaOH at C=0.175 g/dl.
The $\overline{P_n}$ represents the number-average degree of polymerization of PAA.

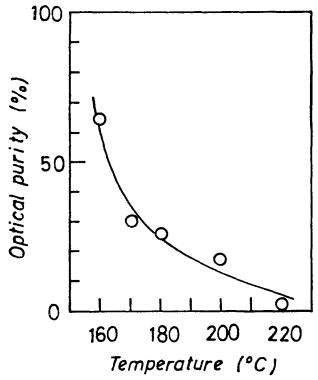


Fig. 3. Temperature effect on the optical purity of APAA.

purity of APAA is shown in Fig. 3. The optical purity decreases rapidly with rise in temperature from 160—180 °C, approaching almost zero percent in the region above 220 °C at which higher yield and higher molecular weight of APAA are obtained. The optical purity of aspartyl residue in the copolymer of aspartic acid and glutamic acid, prepared by thermal polycondensation in the molten state at 170 °C for 3 h, was also almost zero percent.[9] It seems that the racemization of L-aspartic acid by the thermal polycondensation in the molten state takes place faster than that in the solid state.

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