*Effect of Temperature on the Viscosity and the Optical Rotation of Poly-ε-aminocaproyl-α-alanine**

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An interesting property of the synthetic poly-ε-aminocaproyl-α-alanine, an reversible coagulation by heat in acidic or neutral aqueous solution, has been shown by Noguchi and his coworkers[1,2]. In the previous paper[3] the author reported, that the coagulated form of the polymer is much more susceptible than the dissolved form, to the action of trypsin. This fact has been explained by assuming some probable difference which might be present in the structure of both forms of the polymer, although the mechanism of the reversible heat coagulation was not yet clear. Thus, it is interesting to examine the effect of temperature on some physical property of this material, especially the rotatory dispersion for the estimation of the degree of folding of the polymer chain. The present paper deals with the results obtained from experiments on the viscosity and the optical rotation of the D, L- or L-alanine polymer under several temperatures.

Experimental

The preparation method and some properties of poly-ε-aminocaproyl-D, L-alanine (M. W., 21,800) and poly-ε-aminocaproyl-L-alanine (M. W., 7,000) were described previously[3]. The polymer solution was prepared as follows: To 10 ml. of water 100 mg. of the polymer was added and the mixture was stood for over night. After filtration by a glass filter, polymer concentrations in the solution were determined by the method of Kjeldahl N-analysis. pH value of the solution was adjusted by adding 0.2 volume of M/15 phosphate buffer, if it was necessary, small amounts of N HCl or N NaOH were added.

The viscosity was measured by Ostwald's viscosimeter.

The optical rotation of the polymer was determined with the automatic recording polarimeter[4]. Measurements on the optical dispersion were made at a wavelength of 400, 437, 452, 471, 498, 527, 549 and 589.3 mμ; these were obtained from a tungsten lamp and filters except the last one which was the D line of sodium lamp. As sample tube of the polarimeter the capillary cells of 10 cm. in length were used. Temperature control of the polymer solution was carried out by varying the temperature on the water jacket of the sample tube.

Results

The specific viscosity of the polymer was found in an acidic or alkaline media as shown in Fig. 1. A minimum value of the viscosity of the D, L-alanine polymer was found at 55°C in all cases, and was independent on pH and on the concentration of the polymer. The coagulation of the polymer occured at 40 and 50°C in curves I and II respectively, while no coagulation was found in the curve III owing to its high pH value of the medium. This indicates, therefore, that the result given by curves I and II at high temperatures might be meaningless. However, an analogous curve, curve III, was obtained by an

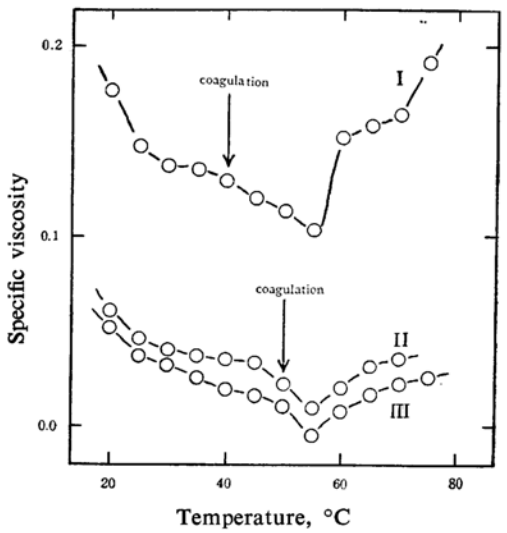

Fig. 1. Effect of temperature on the specific viscosity of the poly-ε-aminocaproyl-DL-alanine.
I: 0.65% polymer, pH 7.1
II: 0.37% polymer, pH 3.7
III: 0.37% polymer, pH 8.7 (no coagulation)
Temperature, ±0.02°C

* This work was presented at the Symposium on Protein Structure, Osaka, October, 1959.

1) J. Noguchi, T. Hayakawa, J. Suzuki and M. Ebata, *J. Chem. Soc. Japan, Pure Chem. Sect.* (*Nippon Kagaku Zasshi*), **76**, 648 (1955).
2) J. Noguchi, T. Hayakawa and M. Ebata, *J. Polymer Sci.*, **23**, 843 (1957).
3) M. Ebata, *J. Biochem.*, **46**, 383 (1959).
4) A. Tsugita, Unpublished (A part appeared in M. Ebata, A. Tsugita and S. Akabori, *Sym. on Enz. Chem. Japan*, 14, (1959), in Japanese).

experiment in the heating process, even under no coagulable conditions. Another experiment in the cooling process agreed with that of the rising one. Curve III shows, that the polymer was transformed to a different type by heating as evidenced by the appearance of a minimum viscosity. The specific viscosity at 55°C in curve III was of a negative value. Thus negative values were often found in the specific viscosity measurement, when the concentrations of the polymer were low although the reason was obscure. Similar curves were also obtained with the viscosity of the L-polymer.

The effect of temperature on the optical rotation of poly-ε-aminocaproyl-L-alanine was studied. The specific rotation of the polymer in aqueous solution was a constant value between 15° to 50°C as shown in Fig. 2. A remarkable increase in *levo*-rotation was found by heating above 53°C. This increase in the rotation was completely reversible with heating and cooling. Addition of a large amount of salts, acids, alkalis and denaturating reagents such as urea did not induce an optical rotation change of the polymer at room temperature, and no coagulation by heat was observed.

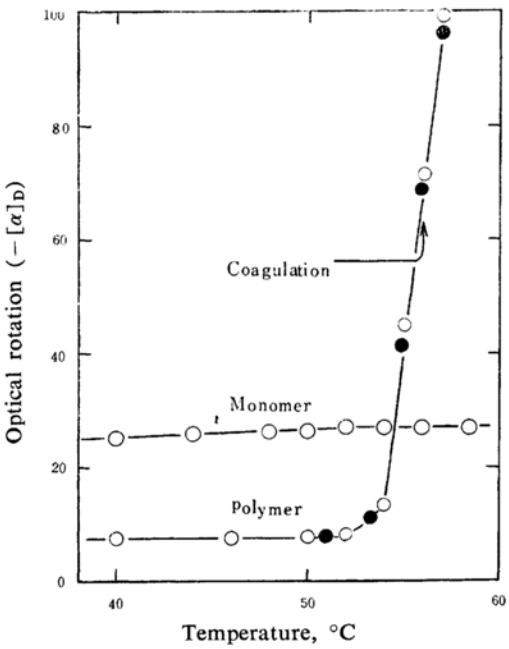

Fig. 2. Optical rotation of poly-ε-aminocaproyl-L-alanine.
Polymer, 0.47% in water (pH 4.0)
Monomer (ε-aminocaproyl-L-alanine), 0.94% in water
○, Points obtained with temperature rising
●, Points obtained with temperature falling
Temperature, ±0.05°C

The rotatory dispersion of the polymer was measured in the aqueous solutions at several different temperatures. When the measurement was made on the solution, its rotatory dispersion was belived to be obeyed Drude's one-term equation, $[\alpha]_\lambda = k_c/(\lambda^2 - \lambda^2_c)$. Where λ stands for the wavelength, $[\alpha]_\lambda$ for the specific rotation at λ, and k_c, λ_c are the rotation constant and the dispersion constant, respectively. In the present experiment the constants were estimated from the data by the usual manner. The λ_c values were 385 mμ (at 30°C), 345 mμ (at 53°C), 315 mμ (at 54°C) and 155 mμ (at 55°C) as can be seen in Fig. 3. No serious coagulation was found at 55°C under the conditions as shown in Fig. 2. Due to the appearance of coagulation, the reasonable value of λ_c could not be estimated from the rotatory dispersion measured at 56°C.

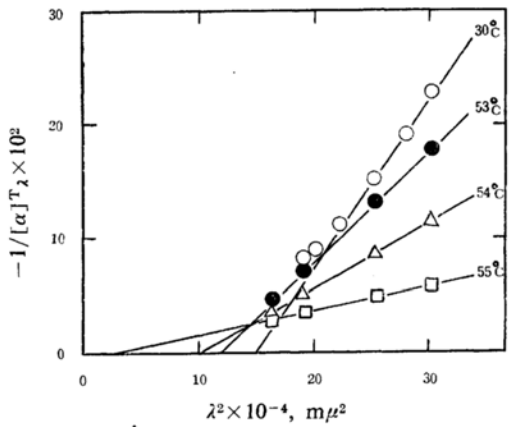

Fig. 3. Rotatory dispersion of poly-ε-aminocaproyl-L-alanine at various temperatures.
Polymer, 0.47% in water (pH 4.0)
Temperature, ±0.05°C

Discussion

The denaturation of protein is known to be accompanied by an increase in specific viscosity and *levo*-rotation and a shift of its λ_c value to shorter wavelength[5,6]. The result on the polymer reported here clearly resembles this information. When the similarity between the protein and the polymer examined here is considered, it is suggested, that a mechanism analogous with protein denaturation might be presented in the nature of the coagulation of the present sample.

According to Doty's results[6,7], a high λ_c value is a measure of the high helix content

5) K. Linderstrøm-Lang and J. A. Schellmann, *Biochim. Biophys. Acta*, **15**, 156 (1954).
6) J. T. Yang and P. Doty, *J. Am. Chem. Soc.*, **79**, 761 (1957).
7) P. Doty and R. D. Lundberg, *Proc. Natl. Acad. Sci.*, **43**, 213 (1957).

of a protein molecule. It is therefore reasonable to assume that the polymer has probably a compact structure in aqueous solution at room temperature and the structure is unfolded by heating. Then, an interesting conclusion described previously[3] that, the coagulated form of the polymer is more susceptible than the dissolved form to the action of trypsin, can be explained by this assumption.

Summary

Viscosity and optical rotation of a synthetic polymer, poly-ε-aminocaproyl-α-alanine, were studied connecting with the reversible heat coagulation. A minimum viscosity of the poly-ε-aminocaproyl-D, L-alanine solution was found at 55°C. Heating of the poly-ε-aminocaproyl-L-alanine solution produces an increase in its *levo*-rotation and a shift of its dispersion constant (λ_c value) to shorter wavelength. The mechanism of the reversible heat coagulation of the polymer was discussed to be based on the obtained result.

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