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NATURE OF ORDERED STRUCTURE OF UNIONIZED POLY-γ-GLUTAMIC ACID STUDIED BY POTENTIOMETRIC TITRATION METHOD

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The potentiometric titration of poly-γ-glutamic acid in aqueous solutions has been studied. The results indicate the conformational transition of this polymer in pH 3 to 5, in agreement with the data obtained by ORD and viscosity measurements. The standard free energy change of the conformational transition ΔG°, standard enthalpy change ΔH° and standard entropy change ΔS° were obtained. The results indicate that the content of the ordered structure in unionized state is small compared with the case of normally presented ordered form of α-polypeptides.

Physicochemical behavior of poly-γ-glutamic acid (γ-PGA) largely depends on the pH of its aqueous solution. Several authors have suggested different conformations for the unionized form and the ionized1)-5), on the basis of the data of ORD and other physicochemical data. In the previous paper3), we have reported that γ-PGA (mixture of 75% D-polymer and 25% L-polymer) of high molecular weight ($\overline{M} = 10^6$) will be mostly a β-form in acid medium (unionized state) and will take a contracted form from the data of ORD, IR spectra(by KBr disc method) and viscosity measurements. However, other authors proposed a different structure such as helix2,4) or hypercoiled form1,5). With the conformation of γ-PGA in ionized state, most authors agreed with each other that γ-PGA took a form of expanded random chain. The ORD curve of unionized γ-PGA shows a Cotton effect at the wavelength below 250 nm3,4,5), whose magnitude of optical rotation is appreciably smaller than that of a 100% α-helix or β-form for α-polypeptides. Furthermore, the difference between the rotational magnitude of a peak (for L-polymer) or a trough (for D-polymer) at 225 nm of the unionized state and that of ionized disordered state at the same wavelength is also small compared with the difference for α-polypeptides. To clarify the cause of these anomalous features of γ-PGA compared with the normally presented nature for α-polypeptides, thermodynamic studies on the conformational transition could provide an useful evidence for the elucidation of the ordered structure of γ-PGA. Therefore, we attempted potentiometric titration of γ-PGA to obtain thermodynamic parameters.

Our sample used in the present work as well as that for our previous work3) was obtained from *B.subtilis No.5E* and it was a mixture of D- and L-polymer. Preparation of pure acid form γ-PGA from a crude material (mixture of sodium salt of poly-γ-D- and poly-γ-L-glutamic acids supplied by Prof. Sawao Murao6) of University of Osaka Prefecture) was made by the similar manner as stated in previous paper3). This purified acid form γ-PGA (mixture of 80% D- and 20% L-polymer)7) was used as the sample. For the present experiment we used two samples which were somewhat different in physical properties: their intrinsic viscosities [η] obtained in dimethylsulfoxide solution at 25° were 0.30 and 1.35 dl/g and the weight average molecular weight of the latter specimen was 5.8×10^4 which was determined from ultracentrifuge measurement by Archibald's method using 0.5 g/dl γ-PGA in 10% NaCl aqueous solution. The both samples were completely soluble in water and NaCl solutions used in present experiment, and the solutions were clear and showed no opalescence after standing. Therefore, the posibility of such a complex formation between D- and L-polymers as stated by Thorne and Leonald$^{8)}$ may be ruled out. The optical rotatory dispersion curve of unionized γ-PGA shows a negative Cotton effect with a trough at 225 nm and the limiting value of specific rotation, $[\alpha]_{225}$ observed below pH 3 is -1500. The magnitude of the corresponding value of $[\alpha]_{225}$ for pure D-polymer is $-1500\times10/6 = -2500$, on the basis of a compensative effect$^{5)}$ on the value of $[\alpha]_{225}$ between D- and L-polymer based on the composition ratio of D:L = 8:2. The value of -2500 is in good agreement with the value of -2660 obtained for acidic form D-polymer by Marlborough$^{4)}$. The change of $[\alpha]_{225}$ with the increase of pH occurred in the range of pH from 3 to 7. Marked increase in solution viscosity with the increase of pH was also observed to occur in the similar range of pH. The magnitude of the limiting value of $[\alpha]_{225}$ did not change with the addition of NaCl, therefore, the possibility of destruction of the ordered structure of unionized γ-PGA in solution would be neglected in the present experiments.

The potentiometric titration was carried out by the aid of Yanagimoto micro burette, and a commercial pH meter under N_2 atmosphere. Similar results were obtained with both samples having different values of [η].

Fig. 1 shows the change of pH as a function of degree of dissociation α. The standard free energy change (per residue) accompanied with conformational transition from the structure at unionized state to that of ionized state was calculated from this figure according to the method of Nagasawa et al.$^{9)}$, i.e. $\Delta G° = 2.3\ RT\cdot A$, where A is the area of the triangle drawn in the figure. Fig. 1 also shows the change of pH as a function of NaCl concentration at 25°. The concentrations of both γ-PGA and NaCl are 3×10^{-3}M for the curve I. When the concentration of γ-PGA only was raised to 7.7×10^{-3}M, the curve agreed well with the curve I, within an experimental error. However, when the concentration of NaCl only was raised to 10^{-2}M (for the curve II), the curve was considerably deviated from the curve I. These results indicate that the effect of added NaCl is greater than that of polymer concentration itself and the addition of NaCl decreased ΔG°.

The value of ΔG° at 25° was plotted against the square root of added NaCl concentration in Fig. 2. The plots were on a straight line and when the concentration of NaCl was zero, ΔG° was 290 cal/residue mole.

The values of ΔG° were plotted against temperature in Fig. 3. The single line drawn was obtained by the least square method applied to the scattered data presented as open circles, equal weight being given to each experiment.

The standard enthalpy change ΔH° was 240 cal/residue mole and the standard entropy change ΔS° = 0.1 e.u. This entropy change is smaller than the value for "the freeze in" of the free rotation in peptide bond (-2.5 – -7.5 e.u.$^{10)}$). The small value of ΔS° together with the small magnitude of $[\alpha]_{225}$ indicates that the

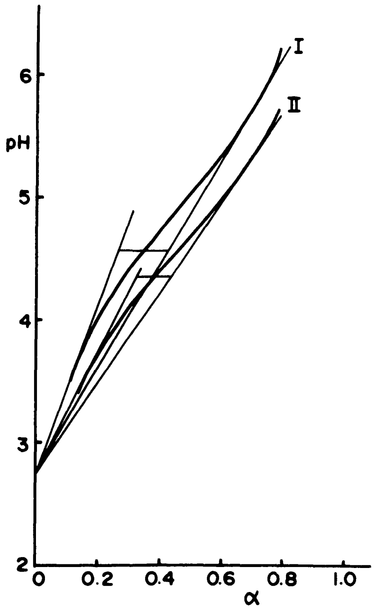

Fig. 1 Titration curves of γ-PGA ([η]=0.30). The straight lines drawn were corresponding to titration curves of ordered structure and disordered structure.
Concn. of γ-PGA, 3×10^{-3}M (residue mole)
Concn. of NaCl, Curve I : 3×10^{-3}M
Curve II : 1×10^{-2}M

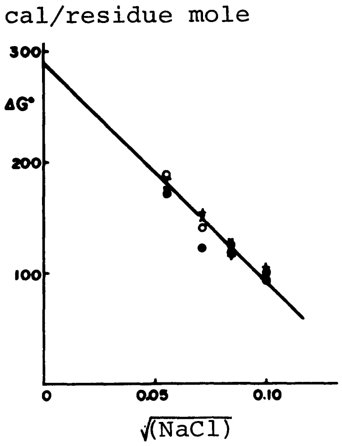

Fig. 2 Standard free energy change, ΔG°, at different concentrations of NaCl at 25°C with γ-PGA of [η]=0.30.
Concn. of γ-PGA in residue mole
+ : 3.00×10^{-3}M
× : 3.87×10^{-3}M
o : 5.79×10^{-3}M
● : 7.70×10^{-3}M

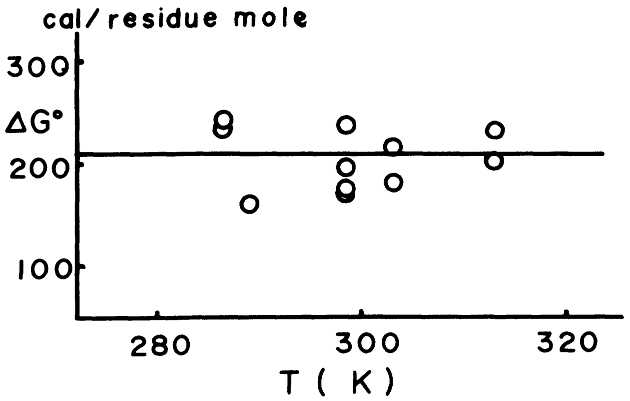

Fig. 3 Free energy change as a function of temperature. Concentrations of γ-PGA ([η]=1.35) and NaCl were 3×10^{-3}M. The single line drawn was obtained by the least square method.

content of ordered structure would be small when the unionized polymer has an ordered structure. Therefore, the conformational transition with the change of the ionization state of the polymer from unionized to ionized one is not a strict one from a structure of high conformational regularity to a disordered structure, but a contracted form having a some regularly hydrogen bonded ordered structure such as β-form or helix, to an expanded random chain.

It was clarified by this experiment that the cause of discrepancy between previous conclusions on the conformation of unionized γ-PGA may be brought about by the fact that the content of ordered structure is small compared with the case of normally presented ordered form of α-polypeptides.

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