

# Helix-Coil 영역에서의 Poly (D-glutamic acid) 의 열적 구조변화에 대한 Poly (vinyl alcohol) 의 영향

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## Effect of Poly (vinyl alcohol) on the Thermally Induced Conformational Change of Poly (D-glutamic acid) in the Helix-Coil Transition Region

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**Abstract:** In relation to denaturation of proteins, thermo-induced conformational change of poly (D-glutamic acid) (PGA) was studied in the presence of poly (vinyl alcohol) (PVA) in the helix-coil transition region. The  $\alpha$ -helix content decreased with annealing time in solution: only a helix-to-coil transition, being independent of the PVA content. The  $\alpha$ -to- $\beta_2$  transition occurred during casting the mixed solution due to the intermolecular hydrogen bonding in the helix-to-coil transition region.

### 1. INTRODUCTION

In the previous paper<sup>1</sup>, the author reported the effect of PVA on the thermally induced conformational change of PGA in the  $\alpha$ -helix region as a model for polypeptide/polysaccharide complex formed in biological systems. It was found that PVA enhanced the  $\alpha$ -to- $\beta_1$  transition of PGA due to intermolecular interaction between the two polymers in a dilute solution and this conformational change was in-

terrupted to a large extent due to the interpenetration of PVA chains into PGA chains which prevented the intramolecular association of PGA chain in a concentrated solution. We are also interested in the thermal behavior of PGA in the presence of PVA during annealing and casting the solution in the helix-coil transition region.

### 2. EXPERIMENTAL PART

Poly(D-glutamic acid)(PGA) : PGA was

prepared from  $\gamma$ -methyl-D-glutamate as the starting material in the following way.  $\gamma$ -methyl-D-glutamate N-carboxy anhydride (D-methyl 3-(2,5-dioxo-1-oxa-3-aza-4-cyclopentyl) propionate) was first prepared by the reaction of  $\gamma$ -methyl-D-glutamate and trichloromethyl chloroformate in dry tetrahydrofuran at 45°C according to the method reported previously<sup>2</sup>. The amino acid N-carboxy anhydride (M) was polymerized in dioxane at 30°C using triethylamine as the initiator (I) at the mole ratio, [M]/[I] of 50. A given amount of the as-polymerized solution was used for the characterization of the resultant poly ( $\gamma$ -methyl-D-glutamate), which was collected as precipitate by pouring the solution into a large amount of methanol. The other portion of the as-polymerized solution was diluted with dioxane to 500cm<sup>3</sup> of 2 wt-% polymer solution, into which 3.64g of sodium hydroxide (1.3 times of the residual moles of poly ( $\gamma$ -methyl-D-glutamate)), which had been dissolved in 30cm<sup>3</sup> of water and 50cm<sup>3</sup> of methanol, was dropwise added with stirring. Immediately the hydrolysis product, poly (D-glutamic acid) sodium salt began to precipitate and the turbidity increased with addition of the sodium hydroxide solution. The reaction was carried out for 24 hr at room temperature. Then the precipitate was collected by filtration, washed with methanol several times and dried in vacuo. About 10 g of PGA sodium salt thus obtained was dissolved in 200cm<sup>3</sup> of water. After filtering the insoluble fraction off, the filtrate solution was adjusted to pH 3 by slow addition of IN-HCl aqueous solution with stirring, which gave rise to the precipitation of PGA. The PGA was collected by centrifugation after keeping the above solution for 24 hr, washed with a small amount of water. This washing and centrifugation were repeated several times, having given rise to gellike PGA, which was then freeze-dried and used for the study. The molar mass of the poly ( $\gamma$ -methyl-D-glutamate) was estimated as  $1.0 \times 10^5$  from the following equation<sup>3</sup> and accordingly the degree of polymerization

was calculated as ca.  $700.[\mu]_{DCA}^2 \frac{5}{M_w} = 2.24 \times 10^{-3} M_w^{0.58}$ , where DCA denotes dichloroacetic acid.

Poly (vinyl alcohol) (PVA): PVA with degree of polymerization of ca. 2000 and OH content of 98.5 mole-% was purchased from Wako Chemicals Co.

Preparation of PVA/PGA mixed solution: 0.2g of PGA was dissolved in 14.2cm<sup>3</sup> of aqueous solution of 0.1 N-NaOH by agitating for 24 hr, which gave rise to pH 7.9-8.0 of the resultant solution and the degree of neutralization of ca. 0.9. Solutions with certain pH's for the subsequent uses were prepared by adding aqueous solution of 0.1 N-HCl to the above mother solution. 100cm<sup>3</sup> of aqueous solution of 2 wt-% PVA was prepared by dissolving the polymer at 80-90°C and used as another mother solution for the subsequent uses. Equal volumes of the PGA solution and the PVA solution, which had been diluted and adjusted to a given pH, were mixed so as to give a constant concentration of PGA independent of the concentration of PVA. The mixing of the two solutions underwent a slight increase of 0.05-0.1 on pH due to dilution of the PGA solution. However, this indicates that PVA did not affect pH when mixing the solution. Thus, the pH of the PGA solution before mixing was adjusted 0.05-0.1 lower than pH to be obtained after mixing.

Heat treatment of the solution of the polymer mixture: The heat treatment was carried out at 105°C in the glass ampule using ca. 0.1cm<sup>3</sup> of the solution for the CD and IR measurements and 20cm<sup>3</sup> of the solution for the viscometry.

IR measurement: The heat-treated solution was cooled for 1hr to room temperature and cast onto a AgCl disk, using ca. 0.15cm<sup>3</sup> of the solution, in a dark room. The IR spectrum was recorded in JASCO IR-G Spectrophotometer under a condition of ca. 95% transmittance at 1900 cm<sup>-1</sup> to estimate quantitatively optical densities of the absorption bands of PGA.

CD measurement: The CD spectrum was recorded for the heat treated and cooled solution

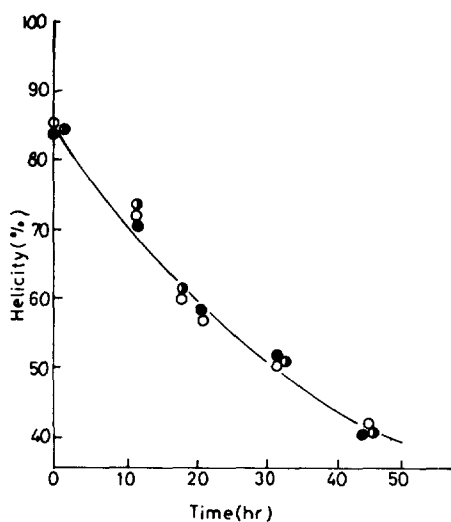


Fig. 1. Percent helicity of poly (D-glutamic acid) (PGA) plotted against the annealing time at 105°C for the pH 5.0 PGA/poly (vinyl alcohol) (PVA) mixed solutions as a function of residual mole ratio,  $[VA]/[GA] = 0/1$  (○),  $0.5/1$  (●) and  $2/1$  (◐). Concentration of PGA was 0.052 mol/l.

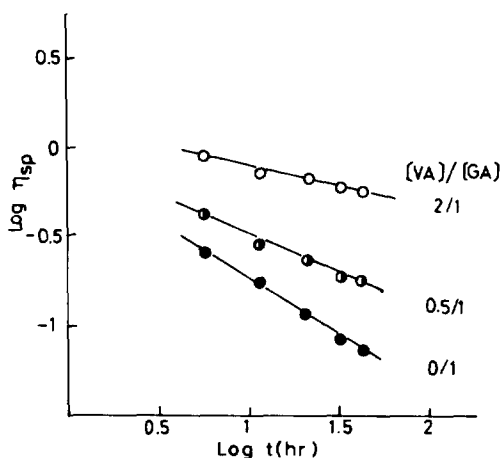


Fig. 2. Logarithmic plots of specific viscosity  $\eta_{sp}$  vs. annealing time  $t$  in h at 105°C for the pH 5.0 poly (vinyl alcohol) (PVA)/poly (D-glutamic acid) (PGA) mixed solutions as a function of residual mole ratio,  $[VA]/[GA]$ . Concentration of PGA was 0.052 unit mol/l.

using a quartz cell with a path length of 0.10mm in a JASCO J-20 Spectropolarimeter.

Viscometry: The viscosity of the heat-treated solution was measured using an Ostwald viscometer at 25°C.

### 3. RESULTS AND DISCUSSION

#### 3-1. The conformation of PGA in the solution

The thermo-induced conformational transition of PGA in the presence of PVA was examined in the helix-coil transition region, at pH 5.0 in the solution with a concentration of PGA of 0.052 residual mol/l. In this solution, the degree of neutralization is 0.42 for PGA and accordingly some of the PGA side groups are dissociated, giving rise to a partially helical conformation. In this solution just after mixing PVA and PGA, i.e., at the annealing time of zero, the  $\alpha$ -helix content of PGA was ca. 85% irrespectively of the  $[VA]/[GA]$  ratio, as is seen from Fig. 1, where the percent helicity estimated from the observed  $[\theta]_{222}$  and the  $[\theta]_{222}$  of  $4 \times 10^4$  (deg) $\times$ (cm<sup>2</sup>) $\times$ (dmol<sup>-1</sup>) for the 100% helicity<sup>4</sup> is plotted against the annealing time at 105°C for the various  $[VA]/[GA]$  values. The helicity decreases with increasing annealing time, irrespectively of the polymer composition. Also the CD spectra indicated the helix to coil transition. Contrary to the effect of PVA on the helix to  $\beta_1$  transition of PGA at pH 4.0, PVA does not affect the thermo-induced conformational transition of PGA at all in the helix-coil transition region. It should be also noted that precipitation of polymers did not occur during the annealing.

Fig. 2 shows the changes of specific viscosity of the solution of the two polymers (the residual concentration of PGA,  $[GA]=0.052$  mol/l) with the annealing time for the various compositions. As seen the specific viscosity decreases with the annealing time in all the cases, reflecting the helix-to-coil transition of PGA. However, one can not draw any conclusion on the state of

mixing of the two polymers or the possible association of the two polymers, since the dependence of the specific viscosity on the polymer composition can be explained simply by the addition of higher molecular weight PVA to PGA.

### 3-2. PGA conformation in cast film

As a specific interaction between PVA and PGA may act during casting the solution, the conformation of PGA was examined by IR measurement for the films cast after annealing the solution. Fig. 3 shows the IR spectra of the cast films, which indicates changes in the absorbances at  $1650\text{cm}^{-1}$  ( $\alpha$ -helix)<sup>5</sup> and  $1608\text{cm}^{-1}$  ( $\beta$ -form)<sup>5</sup> not only with the annealing time before casting but also with the polymer composition. It is also of interest that the IR absorption at  $1630\text{cm}^{-1}$  characteristic of the  $\beta_1$  structure<sup>6</sup> was observed in this case.

The optical density ratio,  $OD_{1608}/OD_{1650}$  estimated from Fig. 3 is plotted against the

annealing time and the polymer composition in Fig. 4 and 5, respectively. It may be said from Fig. 4 and 5 that such an amount of PVA as  $[VA]/[GA]$  of ca. 0.5 enhances the conformational change of PGA from the  $\alpha$ -helix to the  $\beta_2$ -structure in the initial stages of annealing. In case where PVA is rich, on the other hand the  $\beta_2$ -structure formation is retarded by PVA up to the annealing time of ca. 30 h. In the films cast after annealing for more than about 30 h, however, the optical density ratio becomes almost constant irrespectively not only of the annealing time but also of the polymer composition.

### 3-3. Mechanism of the PVA effect on the PGA conformational change

It may be said from the above result that the degree of neutralization of the PGA side chains predominantly affects the difference in the conformation and conformational change of PGA during annealing and casting the solution of PVA/PGA mixture. In fact, the helix-to-coil transition only in the helix coil transition

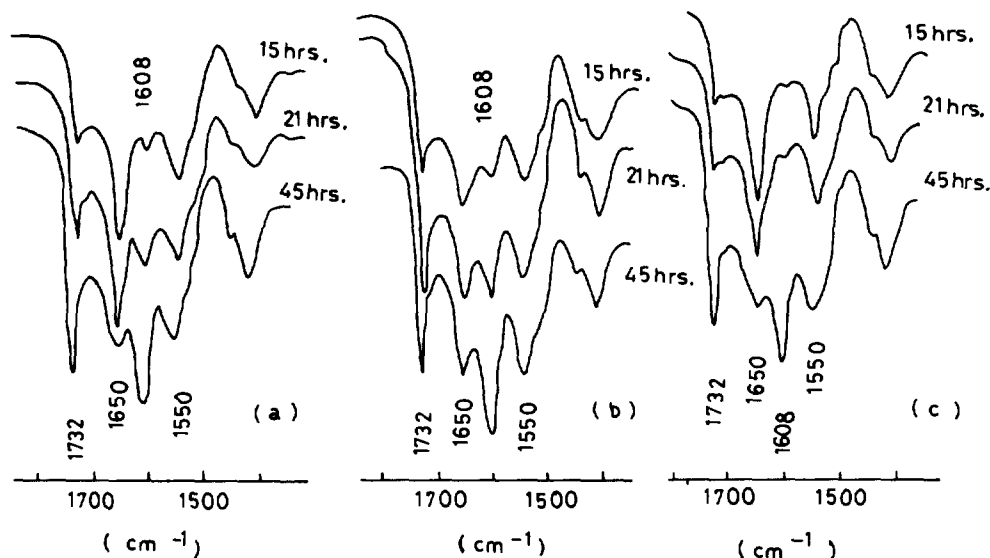


Fig. 3. IR spectra of the films cast after keeping the pH 5.0 poly(vinyl alcohol)(PVA)/poly(D-glutamic acid)(PGA) mixed solutions at  $105^{\circ}\text{C}$  for various times. Concentration of PGA was 0.052 unit mol/l. Residual mole ratio,  $[VA]/[GA] = 0/1$  (a),  $0.5/1$  (b) and  $2/1$  (c).

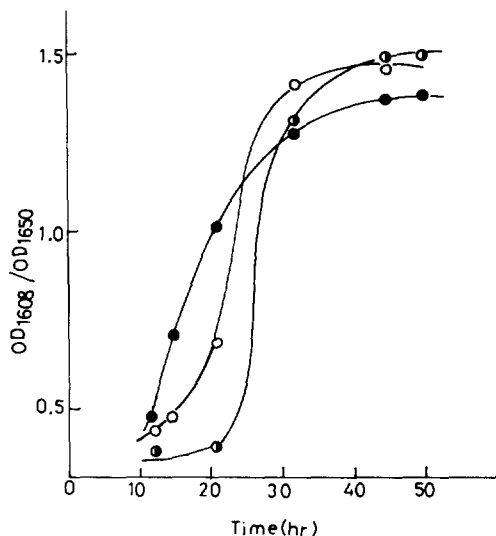


Fig. 4. Plots of optical density (OD) ratio of  $1608\text{cm}^{-1}$  to  $1650\text{cm}^{-1}$  for the films cast after annealing the pH 5.0 poly (vinyl alcohol) (PVA)/poly (D-glutamic acid) (PGA) mixed solutions at  $105^\circ\text{C}$  for various times. Concentration of PGA was 0.052 unit mol/l. Residual mole ratio  $[\text{VA}]/[\text{GA}] = 0/1$  (○),  $0.5/1$  (●), and  $2/1$  (◐).

region of PGA itself, i.e., at pH 5.0, due to the repulsion among the partially ionized carboxyl side groups, irrespectively of the  $[\text{VA}]/[\text{GA}]$  ratio. During casting the solution, however, the PGA chain mainly of random coil seem to convert into the  $\beta_2$  structure. In the PGA-rich system such as of  $[\text{VA}]/[\text{GA}]$  of 0.5/1, the interaction between the PGA molecules and bigger PVA molecules (due to intermolecular hydrogen bonding) may enhance the nucleation of the intermolecular  $\beta_2$  structure of PGA during the casting. In the PVA-rich system such as of  $[\text{VA}]/[\text{GA}]$  of 2/1 the transition in question may be retarded by the existence of PVA due to the fewer chances of the collision between the PGA molecules, the  $\beta_1$  structure being intrinsically intermolecular. In this case, the state of mixing of the two polymers in the solution

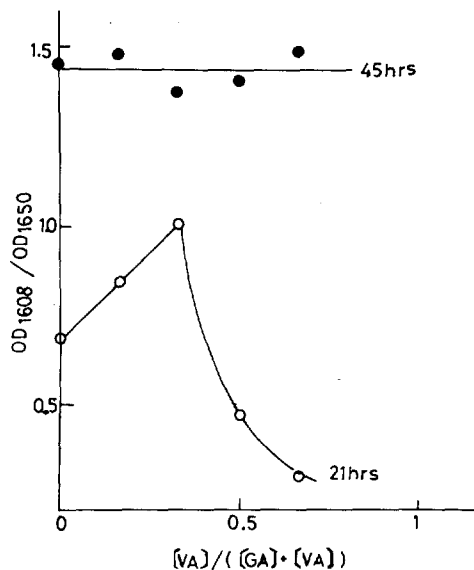


Fig. 5. Plots of optical density (OD) ratio of  $1608\text{cm}^{-1}$  to  $1650\text{cm}^{-1}$  for the films cast after annealing the pH 5.0 poly (vinyl alcohol) (PVA)/poly (D-glutamic acid) (PGA) mixed solutions at  $105^\circ\text{C}$  as a function of annealing time. Concentration of PGA was 0.052 unit mol/l.

affects the transition occurring during the casting.

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