Interaction between Poly(L-ornithine) and Sulfated Poly(vinyl alcohol)

Chong Su CHO, Tadashi KŌMOTO,* and Tohru KAWAI

*Department of Polymer Technology, Tokyo Institute of Technology,
Ookayama, Meguro-ku, Tokyo 152, Japan.*

(Received February 13, 1979)

ABSTRACT: Studies were made on the conformational changes of poly(L-ornithine) (PLO) by interaction with sulfated poly(vinyl alcohol)s (PVS) as functions of pH, temperature, the degree of sulfation and the composition of the polymeric moieties. Polymer-polymer interaction took place below *ca.* pH 10.6, while no interaction of PLO with PVS was found at *ca.* pH 12.0. PLO took on a random coil conformation through complex formations with PVS-25 and PVS-30 (the number indicates the degree of sulfation in mol %) at low pHs. In these complexes with PVS-46 and PVS-95, the α -helix and the β_2 -form of PLO were formed at 1.5°C in the acidic pH region and in the pH region from *ca.* 8.8 to *ca.* 10.5, respectively. The conformation of PLO in the complexes is also discussed in terms of the free-energy change resulting from the conformational change from random coil to the α -helix.

KEY WORDS Polymer Complex / Poly(L-ornithine) / Sulfated Poly(vinyl alcohol) / Circular Dichroism / Conformational Change /

In a previous paper,¹ we studied the interaction between poly(L-lysine) (PLL) and partially sulfated poly(vinyl alcohol)s: PVS-25, PVS-30, PVS-46 and PVS-95 (The number indicates the degree of sulfation in mol%.) as a model for the polypeptide-polysaccharide complex in biological connective tissue. It was found that PLL takes on a random coil conformation in the PLL-PVS-25 complex, a random coil conformation including a small amount of α -helix in the PLL-PVS-30 complex, and the α -helix in the PLL-PVS-46 and PLL-PVS-95 complexes over a wide range of pH, from 2 to 12.

Furthermore, a study was also made on the PLL-PVS-acridine orange complexes to elucidate the structure of the PLL-PVS complexes.² The PLL-PVS-25-acridine orange complex did not give the acridine orange-induced circular dichroism (CD), indicating a random arrangement of the acridine orange molecules bound to the VS groups in the PLL-PVS-25 complex. The PLL-PVS-30-acridine orange complex gave a small acridine

orange-induced Cotton effect. The PLL-PVS-46-acridine orange and PLL-PVS-95-acridine orange complexes gave well-defined induced CD spectra with small troughs at 435 nm and 510 nm and a large peak at 465 nm, indicating a right-handed superhelix of the acridine orange molecules around the right-handed α -helix of PLL chain, where the PVS chain is intercalated. It is probable, however, that the PVS chain segments are arranged in both right- and left-handed superhelices around the PLL helix.

Gelman *et al.*³ studied the interactions between various cationic polypeptides and polysaccharides as functions of side chain lengths of the polypeptides and structures of the polysaccharides.

Since the PVS chain is more flexible than polysaccharide, differences may be found in the interactions of PVS and polysaccharides with polypeptide (*i.e.*, in this case PLO).

In this paper, we discuss the interaction between poly(L-ornithine) (PLO) and PVS, the former having a similar but shorter (by one CH₂) side chain group than that of PLL and taking a conformation with an α -helix content less than that for PLL in aqueous solution at high pH.^{4,5} It is of interest to

* To whom all the correspondences should be addressed.

examine the conformation of PLO in the PLO-PVS complexes formed as functions of the degree of sulfation, temperature, pH, and the conformation of PLO (without any other polymer) in aqueous solution.

EXPERIMENTAL

Materials

A high-molecular-weight PLO ($[\eta]_{\text{DMF}}^{25^\circ} = 1.65 \text{ dl g}^{-1}$) was used as PLO hydrobromide obtained from poly(δ -*N*-carbobenzoxyl-L-ornithine) which had been synthesized from the corresponding amino acid *N*-carboxyanhydride in dioxane at 25°C using triethylamine as initiator. The PVS samples are the same as used in our previous work.^{1,2}

Preparation of PLO-PVA Mixtures in Aqueous Solution

Aqueous solutions of $6.6 \times 10^{-4} \text{ unit mol l}^{-1}$ of PLO and $6.6 \times 10^{-4} \text{ VS-unit mol l}^{-1}$ of PVS, adjusted by HCl and NaOH to the same pH, were mixed by dropwise addition of the PVS solution to the PLO solution with stirring. The concentrations of PLO and PVS were $3.3 \times 10^{-4} \text{ unit mol l}^{-1}$, respectively after mixing.

Circular Dichroism (CD) Measurement

CD spectra of PLL-PVS mixed solutions were recorded in a thermostated quartz cell with a path length of 1.0 mm, using a JASCO J-20 Recording Spectropolarimeter.

RESULTS AND DISCUSSION

pH Dependence of the Conformation of PLO in the PLO-PVS Mixtures at 1.5°C

PLO, whose α -helix is less stable than PLL,^{4,6} undergoes a coil-to- α -helix transition by cooling at a high pH such as *ca.* 11.5. Figure 1 shows the CD spectra of PLO at pH 12.0 at 25°C and 1.5°C , indicating an increase in helical content with decrease in temperature, although the helicity was not so high at 1.5°C as compared with the reported values of *ca.* 100%⁶ and 80%.⁷ It is of interest, therefore, to examine the conformation of PLO in the PLO-PVS mixtures at various temperatures as compared with that of PLL in the PLL-PVS complexes already reported.¹ Figures 2–5 show the CD spectra of PLO-PVS-25, PLO-PVS-30,

Figure 1. CD spectra of poly(L-ornithine) at pH 12.0 and 1.5°C (—), at pH 12.0 and 25°C (-----), and at pH 8.7 and 25°C (- - -).

Figure 2. CD spectra of poly(L-ornithine) (PLO)-25 mol% sulfated poly(vinyl alcohol) (PVS-25) mixtures at 1.5°C as a function of pH. Residue concentration, $[\text{LO}] = [\text{VS}] = 3.3 \times 10^{-4} \text{ mol l}^{-1}$. pH 5.1 (—), pH 9.0 (---), pH 10.4 (- - -), and pH 12.0 (-----).

Figure 3. CD spectra of poly(L-ornithine) (PLO)-30 mol% sulfated poly(vinyl alcohol) (PVS-30) mixtures at 1.5°C as a function of pH. Residue concentration, $[\text{LO}] = [\text{VS}] = 3.3 \times 10^{-4} \text{ mol l}^{-1}$. pH 9.3 (—), pH 10.6 (---), and pH 12.0 (- - -).

Figure 4. CD spectra of poly(L-ornithine) (PLO)-46 mol% sulfated poly(vinyl alcohol) (PVS-46) mixtures at 1.5°C as a function of pH. Residue concentration, $[LO]=[VS]=3.3 \times 10^{-4} \text{ mol l}^{-1}$. pH 5.8 (—), pH 9.1 (---), pH 9.8 (----), pH 10.5 (- - - -), and pH 12.0 (.....).

Figure 5. CD spectra of poly(L-ornithine) (PLO)-95 mol% sulfated poly(vinyl alcohol) (PVS-95) mixtures at 1.5°C as a function of pH. Residue concentration, $[LO]=[VS]=3.3 \times 10^{-4} \text{ mol l}^{-1}$. pH 5.8 (—), pH 8.8 (---), pH 10.5 (----), and pH 12.0 (.....).

PLO-PVS-46, and PLO-PVS-95, respectively as a function of pH at 1.5°C, the temperature at which the mixture solutions were prepared.

In the PLO-PVS-25 (Figure 2), PLO took on an almost random coil conformation at pH 5.1. The $[\theta]$

at *ca.* 198 nm, which is characteristic of a random coil, increased with increasing pH and the CD spectrum at pH 12.0, which is similar to the corresponding spectrum of PLO (Figure 1), indicates a partial α -helix conformation. A similar pH dependency of CD spectrum was also obtained for the PLO-PVS-30 (Figure 3). It may be said from these results that PLO takes on a random coil conformation at 1.5°C below *ca.* pH 10.6 in the PLO-PVS-25 and the PLO-PVS-30 mixtures, and this is in good agreement with PLL-PVS-25 at room temperature.

A great difference in the conformation between PLL and PLO in the mixtures with PVS-25 and PVS-30 was found at high pHs above 11.5. Namely, PLL took on only a random coil formation even at high pHs in the PLL-PVS-25, indicating a strong interaction between PLL and PVS-25, breaking the α -helix of PLL to form the random coil. On the other hand, the formation of the partial α -helix conformation in the PLO-PVS-25 and the PLO-PVS-30 mixtures suggests that there is almost no interaction between the ionic moieties at pH 12.0 and 1.5°C. However, the difference in the value of $[\theta]$ at *ca.* 199 nm between PLO (Figure 1) and PLO-PVS-25 and -30 (Figures 2 and 3) in the neutral and acidic pH regions may suggest that there is an interaction between PLO and PVS-25 and -30. This is plausible since PLO is protonated ($pK_a = 9.8^6$) and PVS-25 and PVS-30 are dissociated as strong polyelectrolytes even at neutral and acidic pHs.

The CD spectra of the PLO-PVS-46 and the PLO-PVS-95 mixtures (Figures 4 and 5, respectively) indicate rather complicated conformational changes occurring with pH. It is of interest that, at pH 5.8 the α -helix of PLO occurred in the mixtures and this helicity was much higher than that for PLO only at pH 12.0 and 1.5°C. The α -helix formation in the PLO-PVS-46 and PLO-PVS-95 complexes at pH 5.8 and 1.5°C is quite similar to those in the PLL-PVS-46 and the PLL-PVS-95.¹ The magnitude of the negative ellipticity, $[\theta]$ at *ca.* 209 nm decreased more than that at *ca.* 222–225 nm with increasing pH, the latter CD trough having been red-shifted up to *ca.* 230 nm. This change in the Cotton effect with pH should correspond to the conformational change from the α -helix to the so-called β_2 -form,^{8–16} since the wavelength of *ca.* 230 nm of the CD trough for the PLO-PVS-46 and the PLO-PVS-95 is close to *ca.* 225 nm for the β_2 -

form. It is noteworthy that the β -structure of PLO has not been reported so far and that the β_2 -form is preferable for PLO since PLL does not take the β_2 -form but the β_1 -form, which has a CD trough at *ca.* 217 nm.¹⁷ In fact, PLL does not take the β_2 -form but the β_1 -form as a minor fraction, the major fraction being the α -helix, in the PLL-PVS complexes.¹

The β_2 -structure of PLO is predominantly formed in pH region around the pK_a of PLO (9.8) in the PLO-PVS-46 and the PLO-PVS-95, being accompanied by formation of a small amount of precipitate which suggests the formation of the intermolecular β -structure. The above result reveals the α -helix-to-the β_2 -form (abbreviation, α - β_2) transition of PLO in the PLO-PVS-46 and PLO-PVS-95 complexes around the neutral pH region at a low temperature such as 1.5°C.

It is also of interest that for the PLO-PVS-46 and PLO-PVS-95, the $[\theta]$ value at *ca.* 230 nm decreased with increasing pH from *ca.* 9 to 10.5 and that the partial α -helix conformation, with helical content considerably lower than that at pH 5.8, could be observed at pH 12.0. This clearly indicates the β_2 -to- α transition in a high pH region such as *ca.* pH 10–11. At pH 10.5, the β_2 -structure was more stable in the PLO-PVS-95 than in the PLO-PVS-46, the latter seeming to have mixed conformations of comparable amounts of the α -helix, the β_2 -form and the random coil. This is probably due to the difference in the charge density between PVS-46 and PVS-95, giving rise to the difference in the strength of the interaction between PLO and PVS. Thus, the β_2 -to- α transition may take place at *ca.* pH 10.5 and pH 11.0–11.5 for the PLO-PVS-46 and the PLO-PVS-95, respectively.

Comparison of the low α -helix content at pH 12.0 with the high content value at pH 5.8 for the PLO-PVS-46 and the PLO-PVS-95 mixtures may indicate that there is no interaction of PLO with PVS-46 and PVS-95 at pH 12.0, since the content value is independent of the charge density of PVS as in the cases of PLO-PVS-25 and PLO-PVS-30, even though PLO without any other polymers takes on a random coil formation at the neutral and acidic pHs and the α -helix at high pHs.

Effect of Temperature on the Conformation of PLO in the PLO-PVS Mixture

Taking into consideration the conformation of PLO sensitive to pH and temperature *via* the charge density of the counter polyion, PVS, examination is made of the conformational stability of PLO in the PLO-PVS mixtures when the temperature is raised from 1.5°C to 25°C. Figures 6–9 show the CD spectra of PLO-PVS-25, PLO-PVS-30, PLO-PVS-46 and PLO-PVS-95, respectively after the mixtures, prepared at 1.5°C, were kept at 1.5°C for 1 h and then at 25°C for 30 min.

As for the PLO-PVS-25, the CD spectra shown in Figures 2 and 6 at the same pH are quite similar to each other except those at pH 12.0. Obviously, this indicates that the strong interaction, *i.e.*, the complex formation of PLO with PVS-25 was maintained when the temperature was increased in the neutral and acidic pH regions. The decrease in the helicity with increasing temperature at pH 12.0, which is quite similar to the α -to-random coil transition by raising the temperature for PLO without PVS-25 (Figure 1), reveals no interaction between PLO and PVS-25, as anticipated from Figure 2. The quite similar phenomena should take place in the PLO-PVS-30 mixture, as evident from

Figure 6. CD spectra of poly(L-ornithine) (PLO)-25 mol% sulfated poly(vinyl alcohol) (PVS-25) mixtures at 25°C as a function of pH. The mixture solution was prepared and kept for 1 h at 1.5°C and then kept for 30 min at 25°C. pH 5.1 (—), pH 9.0 (---), pH 10.4 (----), and pH 12.0 (-·-·-).

Figure 7. CD spectra of poly(L-ornithine) (PLO)-30 mol% sulfated poly(vinyl alcohol) (PVS-30) mixtures at 25°C as a function of pH. The mixture solution was prepared and kept for 1 h at 1.5°C and then kept for 30 min at 25°C. pH 9.3 (—), pH 10.6 (---), and pH 12.0 (.....).

Figure 8. CD spectra of poly(L-ornithine) (PLO)-46 mol% sulfated poly(vinyl alcohol) (PVS-46) mixtures at 25°C as a function of pH. The mixture solution was prepared and kept for 1 h at 1.5°C and then kept for 30 min at 25°C. pH 5.8 (—), pH 9.1 (---), pH 9.8 (.....), pH 10.5 (-----), and pH 12.0 (-----).

comparison of the CD spectra of Figure 7 with those of Figure 3.

As for the PLO-PVS-46, it is evident from Figure 8 that, in the acidic pH region, the α -helix once formed at 1.5°C was not broken when the temperature was raised to 25°C. The α -helix was also maintained at pH 5.8 by raising the temperature for

Figure 9. CD spectra of poly(L-ornithine) (PLO)-95 mol% sulfated poly(vinyl alcohol) (PVS-95) mixtures at 25°C as a function of pH. The mixture solution was prepared and kept for 1 h at 1.5°C and then kept for 30 min at 25°C. pH 5.8 (—), pH 8.8 (---), pH 10.5 (.....), and pH 12.0 (-----).

the PLO-PVS-95 mixture as shown in Figure 9. Comparing the CD spectra of the PLO-PVS-46 mixture at pH 5.8 at 1.5°C (Figure 4) and at 25°C (Figure 8), it may be said that the β_2 -structure existing as a minor fraction decreased and that the α -helix as the major fraction became prominent by increasing the temperature from 1.5°C to 25°C. This suggests a decrease in the number of the intermolecularly associated chains.

In case of PLO-PVS-95 at pH 5.8, the magnitudes of $[\theta]$ at *ca.* 198 nm and *ca.* 222 nm decreased with increasing temperature, indicating that a small fraction of the α -helical PLO changed to the random coil. This may be related to the stability of the α -helix of PLO in the acidic pH region. This tendency was also found at pH 8.8 (Figures 5 and 9).

On the other hand, the β_2 -conformation of PLO in the PLO-PVS-46 mixture was maintained by raising the temperature at pH 9.1 and 9.8, which are below and equal to the pK_a of PLO, respectively. At pH 10.5, change in the temperature gave rise to CD patterns for the PLO-PVS-46 and PLO-PVS-95 mixtures (Figures 8 and 9, respectively), from which an estimation of the conformation is very difficult. This may be partly because of a considerable amount of the precipitates.

The CD spectra of the PLO-PVS-46 (Figure 8) and the PLO-PVS-95 (Figure 9) mixtures at pH 12.0 and 25°C were both similar to that of PLO without

PVS (Figure 1). As was mentioned before, this is also evidence of almost no interaction or a very weak interaction acting between PLO and PVS at pH 12.0. On the other hand, it may be concluded that rather strong interactions are acting between the oppositely charged polymers below pH 9.8, *i.e.*, pK_a of PLO, and even at pH 10.5.

In order to examine the conformational stability of PLO in the PLO-PVS complexes formed at acidic pHs at 1.5°C, CD spectra (though not shown) were taken at various temperatures up to 80°C for the complexes prepared at pH 5.5 and 1.5°C. In the cases of the PLO-PVS-25 and the PLO-PVS-30 mixtures, the CD spectra were very typical of the random coil, since the spectra are independent of temperature. PLO is also in the random coil conformation at pH 5.5 in the absence of any other polymers. But in the case of the PLO-PVS-46 and the PLO-PVS-95 mixtures, the magnitudes of the CD troughs at *ca.* 210 nm and *ca.* 222–225 nm decreased with increasing temperature. As compared with the α -to- β_1 transition of PLL in the PLL-PVS-46 and PLL-PVS-95 complexes with increasing temperature up to 90°C,¹ it may be said that, PLO undergoes conformational changes from both α and β_2 to the random coil with increasing temperature in these cases.

pH Dependence of the Conformation of PLO in the PLO-PVS Mixtures at 25°C

Since the results obtained above suggest that such ordered conformations as the α -helix and the β -form are much less stable for PLO in the complexes with PVS than in the case of PLL, examination should be made of the conformation of PLO in the PLO-PVS mixtures around room temperature. Figure 10 shows the CD spectra of the PLO-PVS-95 mixtures at various pHs, prepared and measured at 25°C. At pH 5.4 and pH 7.3, these mixtures gave CD spectra typical of random coils with the CD troughs at *ca.* 203 nm. However, the magnitude of $[\theta]$ and the wavelength at these troughs are small and red-shifted, respectively, as compared with the CD spectra of the PLO-PVS-25 and the PLO-PVS-30 mixtures with random coil conformations (Figures 2, 3, 6, and 7). The wavelength, *ca.* 203 nm of the CD trough for the PLO-PVS-95 mixture shown in Figure 10 is also different from *ca.* 198 nm for PLO only (Figure 1), even though the CD patterns are similar to each other. These results indicate perhaps

Figure 10. CD spectra of poly(L-ornithine) (PLO)-95 mol% sulfated poly(vinyl alcohol) (PVS-95) mixtures at 25°C as a function of pH. The mixture solution was prepared at 25°C. $[LO]=[VS]=3.3 \times 10^{-4} \text{ mol l}^{-1}$, pH 5.4 (—), pH 7.3 (---), pH 9.6 (----), and pH 11.7 (.....).

that PLO forms a complex with PVS-95, and probably with the other PVS samples as well, in the acidic pH region at 25°C. It is very clear, however, that, even if the PVS-95 chain is strongly associated with the PLO chain in a random coil, no transition to an ordered conformation takes place at all.

The great difference in the conformation of PLO in the PLO-PVS-95 (probably in the PLO-PVS-46 mixture as well) between at 1.5°C (Figure 5) and at 25°C (Figure 10) may be accounted for by the opposite signs of ΔG^0 , the free energy of the conformational transition from coil-to-helix: *i.e.*, at a low temperature such as 0.8°C, $\Delta G^0 = -28 \pm 2 \text{ cal mol}^{-1}$ and at 25°C, $\Delta G^0 = \text{positive}$ for PLO only.⁶ The positive ΔG^0 for PLO at and above 25°C can also account for the conformational changes from both the α -helix and the β_2 -form to random coil at high temperatures for the PLO-PVS-46 and the PLO-PVS-95 mixtures mentioned above. The α -helix formation of PLL in the PLL-PVS-46 and the PLL-PVS-95 complexes at acidic pHs¹ agrees well with large negative values of ΔG^0 such as $-138 \text{ cal mol}^{-1}$ at 25°C and $-168 \text{ cal mol}^{-1}$ at 0.5°C.⁶

Effects of the Residue Mole Ratio, $[VS]/[LO]$ on the Helicity of PLO

In order to clarify the structure of the complex of PLO with PVS, especially in the case of the α -helix at low pHs, $[\theta]_{222}$ was measured by changing the residue mole ratio, $[VS]/[LO]$. Quite similarly to the

PLL-PVS-46 and the PLL-PVS-95 complexes,¹ $[\theta]_{222}$ decreased with increasing $[\text{VS}]/[\text{LO}]$ and became constant (ca. $-1.5 \times 10^4 \sim -1.7 \times 10^4$ deg cm² dmol⁻¹ of LO residue) for $[\text{VS}]/[\text{LO}] \geq 1$. This is the reason why $[\text{VS}]/[\text{LO}]$ of 1 was chosen for the CD measurements for examining the conformation of PLO in the PLO-PVS mixtures. Consequently, the highest helicity attained at $[\text{VS}]/[\text{LO}] \geq 1$ (although a small amount of the β_2 -form was contained) is very similar to the $[\text{VS}]/[\text{LL}]$ dependency of PLL conformation for the PLL-PVS-46 and the PLL-PVS-95 complexes at low pHs, suggesting that the PVS chain is so flexible that it can undergo large conformational changes following conformations of PLL or PLO itself from the random coil to the α -helix.

REFERENCES

1. C. S. Cho, T. Kōmoto, A. Nakagami, and T. Kawai, *Makromol. Chem.*, **180**, 1951 (1979).
2. C. S. Cho, T. Kōmoto, and T. Kawai, *Makromol. Chem.*, **180**, in press (1979).
3. R. A. Gelman and J. Blackwell, *Biopolymers*, **13**, 139 (1974).
4. G. Blauer and Z. B. Alfassi, *Biochim. Biophys. Acta*, **133**, 206 (1967).
5. S. R. Chaudhuri and J. T. Yang, *Biochemistry*, **7**, 1379 (1968).
6. M. J. Grouke and J. H. Gibbs, *Biopolymers*, **10**, 795 (1971).
7. Y. C. Fu, H. E. Van Wart, and H. A. Scheraga, *Biopolymers*, **15**, 1795 (1976).
8. G. D. Fasman and J. Potter, *Biochem. Biophys. Res. Commun.*, **27**, 209 (1967).
9. L. Stevens, R. Townend, S. N. Timasheff, G. D. Fasman, and J. Potter, *Biochemistry*, **7**, 3717 (1968).
10. H. Maeda and S. Ikeda, *Biopolymers*, **10**, 1635 (1971).
11. T. Imae and S. Ikeda, *Biopolymers*, **11**, 509 (1972).
12. C. Toniolo and G. M. Bonora, *Makromol. Chem.*, **175**, 2203 (1974).
13. C. Toniolo and G. M. Bonora, *Makromol. Chem.*, **176**, 2547 (1975).
14. C. Toniolo and G. M. Bonora, *Biopolymers*, **13**, 2179 (1974).
15. T. Hayakawa, Y. Kondo, and Y. Murakami, *Polym. J.*, **6**, 424 (1974).
16. K. Itoh and G. D. Fasman, *Biopolymers*, **15**, 419 (1976).
17. R. Townend, T. F. Kumosinski, S. N. Timasheff, G. D. Fasman, and B. Davidson, *Biochem. Biophys. Res. Commun.*, **23**, 163 (1966).