Neutral Salt Effect on the Interaction of Poly-α,L-glutamic Acid with Acridine Orange

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The effect of potassium chloride on the absorption and optical rotatory properties of the poly-α,L-glutamic acid-acridine orange system in an aqueous solution was studied under various conditions. The stability of the helical conformation of poly-α,L-glutamic acid in this system was examined at different potassium chloride concentrations by estimating the helix fraction of the polymer. At pH 4.5, where poly-α,L-glutamic acid exists in the helix, it was found that the added potassium chloride raised the stability of the helical conformation of the polymer in this system. In this case, it was assumed that the added potassium chloride lowers the disturbance of helix formation by the bound acridine orange cation. However, when the solution's pH was raised, the added potassium chloride lowered the extent of the helix fraction in this system. These phenomena were discussed on the basis of several experimental findings on the circular dichroism of the systems. Furthermore, from the change in the intensities of the circular dichroism with the variation in the potassium chloride concentration, it was presumed that the positive circular dichroism band at about 520 nm was due to the binding of dimeric acridine orange molecules to the polymer.

Many investigators[1-3] have studied the interaction of biopolymers, such as polynucleotides,[4,5] or polypeptides,[6,7] with dyes. It is well known that an extrinsic optical activity is induced in the region corresponding to the absorption bands of acridine orange (AO) only when the dye combines with the helical form of poly-α,L-glutamic acid (PLGA) in an acidic solution. Furthermore, in the course of studies of the interaction of PLGA with AO under conditions of various pH or R/D (the ratio of glutamyl residues to dye molecules) ratios,[8,9] we found that, in the case of a R/D of around unity, the induced Cotton effects can also be observed even when the AO molecules are bound to PLGA in the neutral and alkaline pH region.[8] In this case, it was confirmed that the PLGA–AO complex had a helical conformation. Thus, extrinsic optical activity can be expected in the aqueous solution system of PLGA–AO, in which PLGA forms a helix.[10] Ballard *et al.*[2] assigned the induced circular dichroism (CD) bands of the PLGA–AO complex in an aqueous solution by measuring the CD spectrum of the complex under streaming conditions. Yamaoka and Resnik[11] discussed the optical activity in this system at very large R/D values. They proposed that the induced Cotton effects could arise from monomeric dye bound near the asymmetric environment of the α-carbon atom of the peptide residue.

In this paper, we will discuss the effect of potassium chloride on the interaction of PLGA with AO. The origin of the CD band at 520 nm will also be considered on the basis of the effect of potassium chloride at small R/D values, and will be compared with the dimer formation of AO.

Experimental

Materials. The sodium poly-α,L-glutamate used here was supplied by the Ajinomoto Co., Inc. The degree of polymerization of the polymer was 650. The AO was purified according to the method described in previous paper.[9]

Procedure. The mixtures were prepared by adding an aqueous AO solution to an aqueous PLGA solution with a pH of 4.5 in the presence or absence of potassium chloride, and then the pH of the mixed solutions was brought to the desired value by the addition of 1/10N NaOH. The R/D value was varied from 1 to 10, but the final concentration of AO was fixed at 2×10^{-5} M. The spectra of all the mixed solutions were measured within a few hours after their preparation. The CD and absorption spectra were measured by means of a JASCO Model ORD/UV-5 dichrograph with a CD attachment and by means of a Hitachi Model EPS-3T spectrometer respectively, both times at room temperature. The pH values of the solutions were measured by means of a Toa Denpa Kogyo Model HM-8 pH meter under a streaming of nitrogen gas. All the data presented below are reduced to the molar basis of the total dye concentration or the glutamyl residue concentration, and are expressed by the molar extinction coefficient, ε, and the molar ellipticity, $[\theta]$. As for the dependence of the concentration on the ellipticities, we refer to each figure.

Results and Discussion

Effect on Absorption Spectra. AO molecules aggregate in an aqueous solution. Thus, in the concentration range from 10^{-3} to 10^{-6} M of AO, as the concentration increases, the intensity of the monomer band at 492 nm (α-band) gradually decreases and the dimer band at about 470 nm (β-band) appears.[12]

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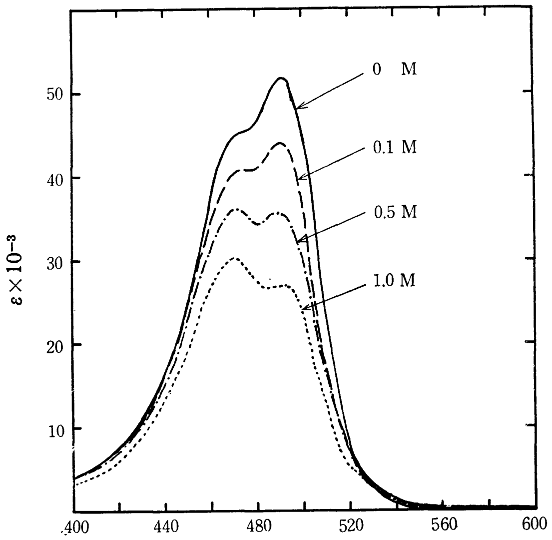

Fig. 1. Absorption spectra of AO at various KCl concentrations (pH=4.5, [AO]=2×10^{-5}M).

With a further increase in the concentration of AO, the β-band shifts toward a shorter wavelength (γ-band, about 450 nm); this corresponds to the formation of higher aggregates.

Figure 1 shows the change in the absorption spectrum of AO with a variation in the potassium chloride concentration. As the concentration of potassium chloride increases, the intensity of the monomer band becomes weak, while the dimer band increases in intensity. There is an isosbestic point at about 520 nm, indicating that only two molecular species, the AO monomer and the dimer, are present in the system. The AO dimer is probably held by dispersion force and/or hydrophobic interaction between the π-electron system, which overcomes the electrostatic repulsion force between the two AO cations. From Fig. 1, it is obvious that potassium chloride promotes the dimerization of AO. This may be due to the fact that the binding of the chloride anions to the AO cation reduces the electrostatic repulsion force between the cations of AO. The coulombic repulsion between the AO cations is minimized when the charged amino groups on both sides lie along opposite edges of the sandwich,[13] and the anions are attached laterally to the dye cation without affecting the internal coupling of AO. Therefore, it is reasonable that Fig. 1 shows the extent of the dimerization of the AO cations.

If the association is enhanced through an increase in the concentration of Cl$^-$, the following two equilibria can be considered for the association step of the AO cations in the presence of the anion:[14]

$$2D^+ + A^- \rightleftharpoons (D_2A)^+ \tag{1}$$

$$2D^+ + 2A^- \rightleftharpoons (D_2A_2) \tag{2}$$

where D$^+$ and A$^-$ denote the dye cation and the anion respectively.

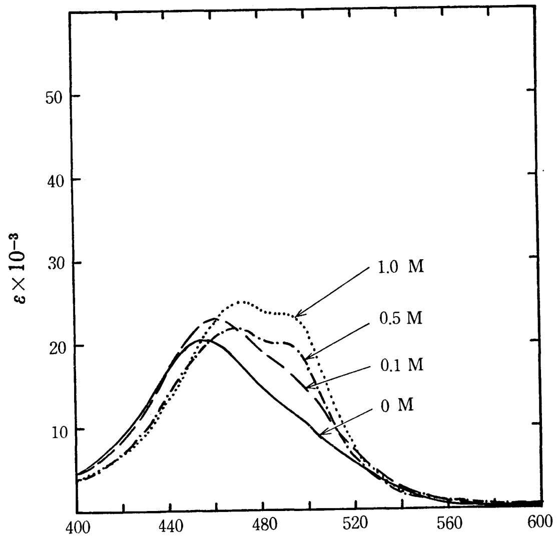

Fig. 2. Absorption spectra of PLGA–AO system at various KCl concentrations (R/D=10, pH=4.5, [AO]=2×10^{-5}M).

Figure 2 shows the change in the absorption spectrum of AO in an aqueous solution with various potassium chloride concentrations at pH=4.5 and R/D=10. The intensity of the γ-band is stronger than those of the α-band and the β-band; however, with an increase in the potassium chloride concentration the intensity of the β-band becomes rather strong. This spectral profile is scarcely influenced by the variation in R/D values. From these results, it can be concluded that the addition of potassium chloride to the AO system promotes the dimerization of the AO cation, even in the presence of PLGA.

Effect on the Helix-coil Transition of PLGA. Figure 3 shows the helix-coil transition of PLGA at various potassium chloride concentrations. The helix-coil transition of PLGA occurs in the pH range from 5.6 to 6.8. However, the transition range of pH was gradually lowered with an increase in the potassium chloride con-

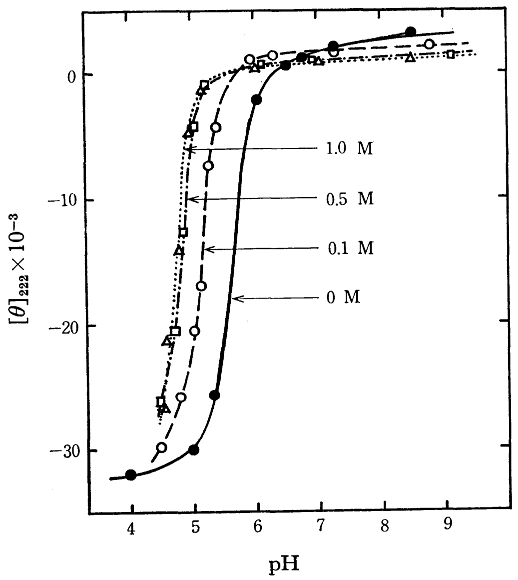

Fig. 3. Helix-coil transition of PLGA at various KCl concentrations.

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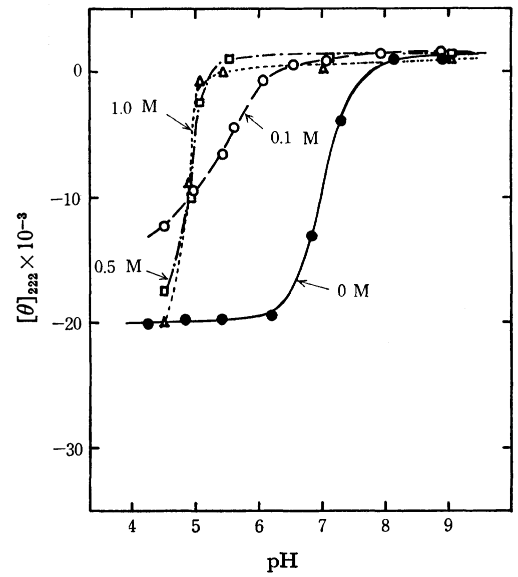

Fig. 4. Helix-coil transition of PLGA in PLGA–AO system at various KCl concentrations (R/D=6, $[AO]=2\times10^{-5}M$).

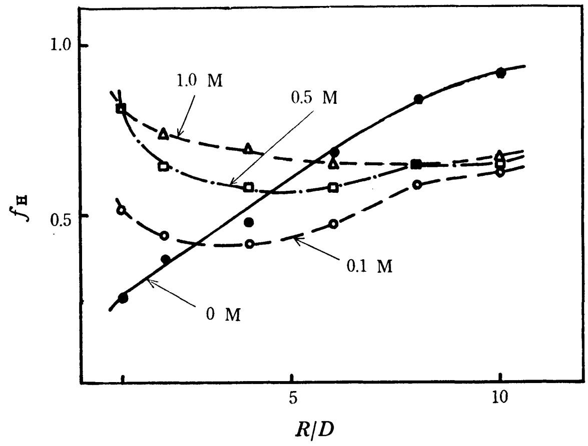

Fig. 5. R/D dependence of helix fraction (f_H) of PLGA in PLGA–AO system at various KCl concentrations (pH=4.5, $[AO]=2\times10^{-5}M$).

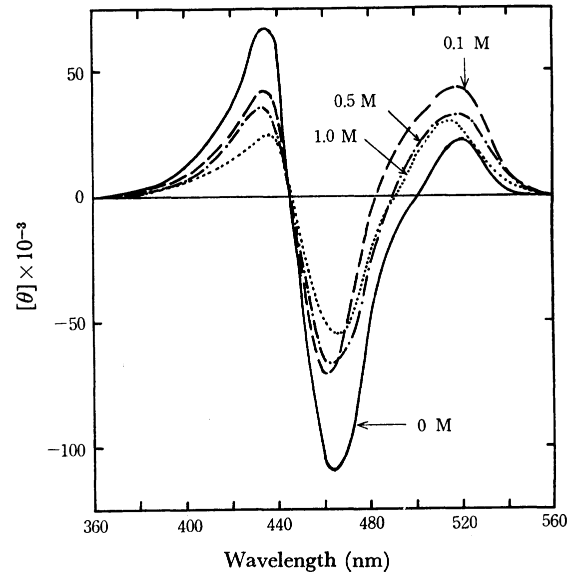

Fig. 6. CD spectra of PLGA–AO system at various KCl concentrations (R/D=10, pH=4.5, $[AO]=2\times10^{-5}M$).

centration. This indicated that the apparent pK_a value of PLGA was lowered by the electrostatic interaction between the carboxylate anions of side chains and the potassium cations.[15]

Figure 4 shows the effect of the addition of potassium chloride on the helix-coil transition of PLGA in the PLGA–AO system. At lower concentrations of potassium chloride, the transition range of pH remained unchanged, as dose the range in the case of the PLGA solution. When the R/D values are small, *e.g.*, smaller than about 10, the AO cations come to bind to the carboxylate anions of PLGA with an increase in the pH value in the absence of potassium chloride and stabilize the helical structure of PLGA.[9]

On the contrary, the results in Fig. 4 show that, at higher concentrations of potassium chloride, the stabilization of the helical structure of PLGA by AO molecules is lowered. In this case, this phenomenon may be elucidated by the fact that the neutral AO dimer is formed according to Eq. (2), thus reducing the binding of AO cations to PLGA.

At various concentrations of potassium chloride in the PLGA–AO system, the helix fraction (f_H) of PLGA was estimated in order to examine the influence of potassium chloride on the helix stability of PLGA (Fig. 5). It was found that f_H values tended to increase with a decrease in the R/D values when potassium chloride was added to the PLGA–AO system. This implies that the decrease in f_H value results from reducing the ionic coupling of the AO cation with the carboxylate anion. Therefore, the interaction of PLGA with AO is lowered and the stability of helical structure increases with a decrease in the R/D value and with an increase in the concentration of potassium chloride.

Relation between Dimer Formation and CD. The magnitude of CD is also dependent on the potassium chloride concentration, as may be seen in Fig. 6. While the magnitude of the positive CD band at about 435 nm and that of the negative CD band at about 465 nm decrease with an increase in the potassium chloride concentration, the magnitude of the positive CD band at about 520 nm increases with an increase in the concentration of potassium chloride. This spectral change is elucidated by the fact that, at higher concentrations of potassium chloride, the dyes aggregated in a helical fashion along the peptide chain decrease. The extent of the increase in the ellipticity of the CD band at 520 nm is larger when the potassium chloride concentration is lower. Since Eq. (1) is applicable in the case of lower concentrations of potassium chloride, where the cationic AO dimer is easily formed, AO dimer can be bound to the ionized carboxyl groups of PLGA. At higher concentrations, however, Eq. (2) seems to be more reasonable; if it holds, the binding of the AO dimer would be reduced. The change in the ellipticity of the CD band at 520 nm can be explained as follows. From the correlation between the positions of the absorption bands of the AO dimer, it can be demonstrated that the CD band at 520 nm is

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due to the binding of the dimeric AO molecule to the isolated site in an asymmetric environment of PLGA. Thus, the positive CD band at 520 nm can be ascribed to the Cotton effect originating from the AO dimer bound to PLGA. This assumption is consistent with the information obtained from the electronic spectrum of the AO dimer.[12,16,17]

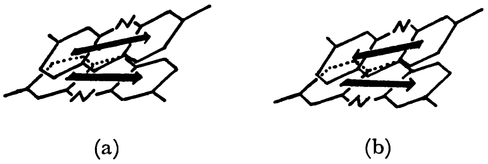

Fig. 7. AO dimer on PLGA–AO complex. The arrows are direction-pair of the monomer transition dipoles.

The dimer spectrum of the AO molecule has two peaks, a strong one at about 465 nm and a weak one at about 510 nm, which can be explained in terms of the simple exciton theory.[18] The stronger band at the shorter wavelength can be assigned to an allowed transition, and the weaker band at the longer wavelength, to a forbidden transition. When AO molecules in the dimer state are oriented at small angles to each other in binding to PLGA, an optical activity is induced and the CD band arising from the dimer on PLGA may split into two components, having signs opposite to each other.

In the staggered conformation for the dimeric AO, we can consider two modes for the direction-pair of the monomer transition dipoles: (a) the directions of the monomer transition dipoles are the same, and (b) the directions of the monomer transition dipoles are opposite to one another. In the case of (a), a negative CD band arises at a shorter wavelength, whereas a positive CD band arises at a longer wavelength in the case of (b). The weak negative dichroism, concealed under the larger negative CD band, can be expected at a shorter wavelength, besides a positive dichroism at 520 nm.

Yamaoka and Resnik[11] proposed that the induced Cotton effects could arise from the dye monomers bound near the asymmetric carbon atoms in polypeptide in the case of very large R/D values. Under these conditions, the Cotton effects may be induced mainly by an interaction between dyes in the monomeric state on PLGA, since the aggregation of dye on the polymer is not plausible. On the other hand, in the case of smaller R/D values the Cotton effects may be induced mainly by an exciton interaction between dye dimers bound to PLGA.

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