

Conformational studies of basic poly(α -amino acid)s in reversed micelles

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Abstract: The conformation of various basic poly (α -amino acid)s was investigated by CD measurements in aqueous solutions containing bis (2-ethylhexyl)sodium sulfosuccinate (AOT) as well as in the AOT reversed micelles. The addition of AOT into an aqueous solution of poly(L-lysine) induces the conformational transition from coil to ordered structure, followed by aggregation. On the other hand, poly(L-lysine) assumes β -structure in the reversed micelles at low w_0 value ($w_0 = [\text{H}_2\text{O}]/[\text{AOT}]$). Similarly to poly(L-lysine), poly(L-ornithine) takes an ordered structure in the aqueous solution containing AOT and β -structure in the reversed micelles. In this case, however, these ordered structures are not so stable, compared with that of poly(L-lysine). Poly(L-arginine) undergoes the conformational transition from coil to helix by addition of AOT into the aqueous solution. Further addition of AOT allows transformation into β -structure. Copoly(L-lysyl-L-leucine) with 63 % leucine residue was shown to take a stable helical conformation even in pure water. In the reversed micelles, however, this ordered structure is significantly changed probably because the hydrophobic interaction among the leucyl residues is lowered in the reversed micelles.

Key words: Reversed micelle, Basic poly(α -amino acid)s, Circular dichroism measurement, and Conformational transition.

Introduction

It is well known that poly(α -amino acid)s carrying ionic side chains does not obtain ordered structures in aqueous solutions. This is usually attributed to the strong electrostatic repulsion. The ordered structure of these polymers is, therefore, induced by the electrostatic shielding effect and/or the destruction of the hydration shell. For example, the ordered structure of basic homopoly(α -amino acid)s such as poly(L-lysine) is induced by addition of water structure breaking anions like ClO_4^- [1]. On the other hand, the addition of water structure making anions like SO_4^{2-} does not induce the order formation probably because of the extended hydration shell of this anion which prevents a specific interaction with the cationic groups of the side chains, as has been reported [1, 2, 3]. It was shown that the addition of organic solvents like alcohol to aqueous solutions of ionic homopoly(α -amino

acid)s leads to a conformational transition from coil to helix [4, 5, 6, 7]. This finding suggests that a change of the dissociation constant diminishing the electrostatic repulsion, and maybe the hydration shell due to ion-dipole interactions around the charged side chains, are to some extent responsible for the helix formation, and indicates that the replacement of electrostricted water molecules by organic molecules causes the conformational transition into α -helix. Moreover, the anionic surfactants like sodium dodecyl sulfate (SDS) are known to stabilize β -structure of poly(L-lysine) or α -helix of poly(L-ornithine) [8]. Such a structure inducing effect is owing to the long hydrophobic hydrocarbon chains of these surfactants.

In the case of poly(L-arginine), an α -helical conformation is induced by the addition of SDS to aqueous solutions [9].

As mentioned above, the ordered structure of basic poly(α -amino acid)s are induced by addition of alcohol, salt, or surfactant, and the situation is somewhat different in each case. In the present work, basic homopoly(α -amino acid)s, i.e., poly(L-lysine), poly(L-

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ornithine), and poly(L-arginine), and copoly(L-lysyl-L-leucine) were studied in the reversed micelles of bis(2-ethylhexyl) sodium sulfosuccinate (= AOT) in octane as well as in aqueous solutions containing AOT, focusing on the change of the intermolecular interactions among side chains.

Experimental

Materials

Basic homopoly (α -amino acid)s

Poly(N^{ϵ} -carbobenzoxy-L-lysine) and poly(N^{δ} -carbobenzoxy-L-ornithine) were prepared by polymerizations of the corresponding NCA's with triethylamine as an initiator, respectively. Poly(L-lysine) and poly(L-ornithine) were obtained by removal of the protecting groups of the corresponding polymers according to the usual way [10]. Poly(L-arginine) was obtained by guanidization of poly(L-ornithine) with 3,5-dimethylprazolyl formamidinium nitrate.

Copoly(L-lysyl-L-leucine)

Copoly(L-lysyl-L-leucine) was prepared by decarboxylation of copoly(N^{ϵ} -carbobenzoxy-L-lysyl-L-leucine) as shown in the previous work [11].

Bis(2-ethylhexyl)sodium sulfosuccinate (AOT)

AOT was purchased from Wako and purified as described in the article [12].

Method

A certain amount of aqueous polymer solution was added into an 0.05 M AOT/octane mixture with vigorous stirring, where the polymer concentration in mixed solutions ranged from 1.84×10^{-4} to 2.76×10^{-4} (M). The mixed solutions were sufficiently shaken prior to the CD measurements.

The conformations of the polymers in aqueous solutions containing AOT or sodium ethanesulfonate were also measured.

Measurement

Circular dichroism measurements (CD) were carried out using a Jasco spectropolarimeter model J-20.

Results and discussion

Figure 1 shows the CD spectra of poly(L-lysine) in aqueous solutions containing AOT. The addition of AOT enhances to take an ordered structure. The shape of the CD spectra seems to be a superposition of α -helix and β -structure. Further addition of AOT leads to a decrease in the magnitude of $[\theta]_{222}$.

The relationship between $-[\theta]_{222}$ and AOT concentration was inserted into Figure 1, since the change in $[\theta]_{222}$ qualitatively refers to the change of the helical

content. The CD spectra observed in the present work are similar to the spectra for the aggregation of α -helices suggested by Yang or Bello [15,16]. This decrease seems to be attributed to aggregation of polymer molecules, concomitantly with precipitation. It is considered that this helix-inducing effect is due to the hydrophobic interaction between the hydrophobic part of the side chain and the methylene groups of AOT as well as the depression of electrostatic repulsion between cationic side chains. In order to examine the contribution of electrostatic interactions to the helix formation, the conformational behavior was studied by addition of sodium ethanesulfonate to an aqueous solution of poly(L-lysine). As becomes obvious from Figure 2, any ordered structure is not observed. The helix-inducing effect of AOT seems to be due to both contributions: one of them is the electrostatic interaction between the anionic group of AOT and the cationic group of the side chain of polymer, but this is not enough to induce an ordered structure. Another is the hydrophobic interaction between the long methylene chains of AOT molecules, which are oriented

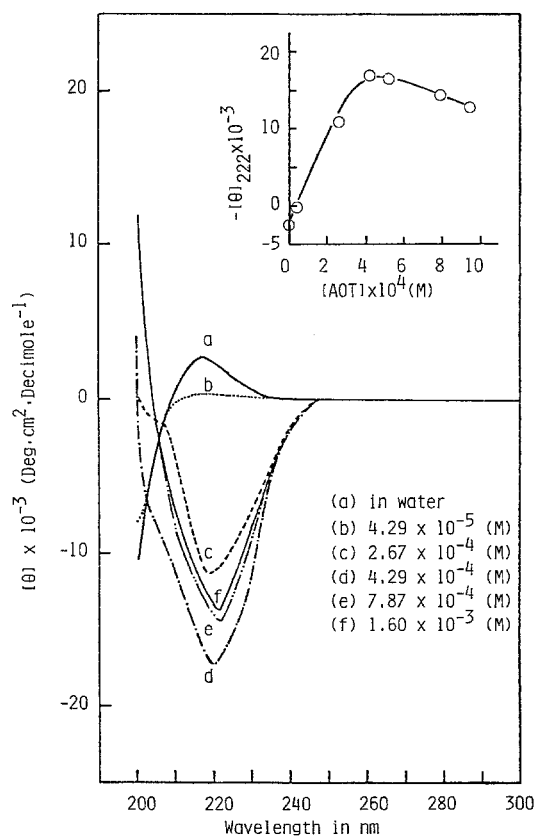


Fig. 1. CD spectra of poly(L-lysHBr) in water and AOT solutions at $\text{pH} = 5.9 \pm 0.2$

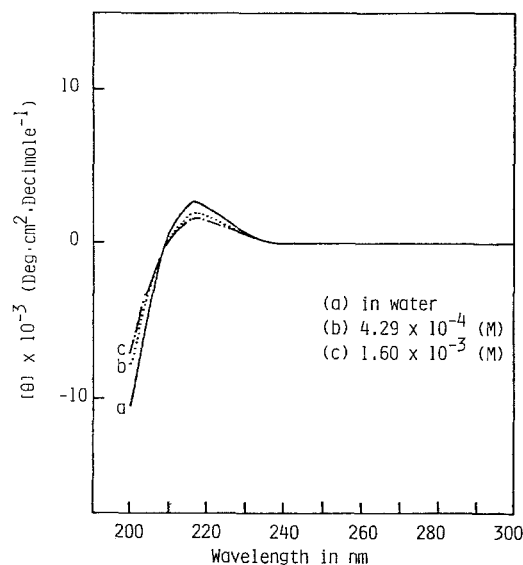


Fig. 2. CD spectra of poly(L-lysHBr) in water and sodium ethanesulfonate solutions at pH = 5.8 ± 0.1

along the polymer chain owing to the electrostatic interaction. The situation is shown schematically in Figure 3.

Figure 4 shows CD spectra of poly(L-lysine) in the reversed micelles at various w_0 values ($w_0 = [H_2O]/[AOT]$). Judging from the CD spectra, poly(L-lysine) is considered to take the β -sheet structure in the reversed micelles. The formation of the ordered structure is promoted as the w_0 value becomes smaller. The water molecules in the reversed micelle are known to be in two different states. The water molecules which contact with the hydrophobic groups of AOT are in the hydrated and restricted state, and the water molecules far from the hydrophobic groups are in the free

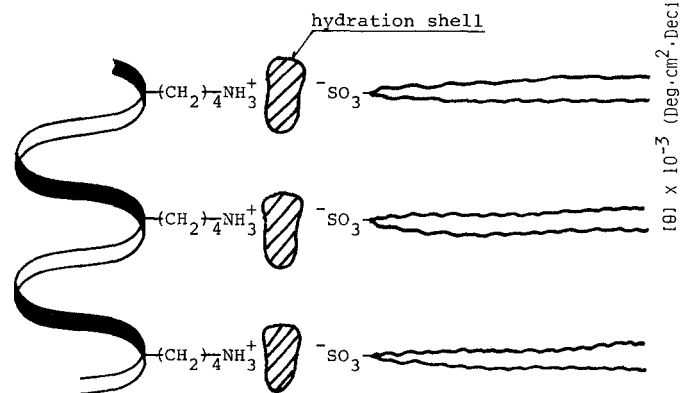


Fig. 3. Schematic profile exhibiting the interaction between the side chains of poly(L-lysine) and the AOT molecules.

state [13, 14]. The relative ratio of the free water molecules decreases as the w_0 value becomes smaller. The order-formation effect in the reversed micelles could be interpreted based on this consideration as follows: the hydration shell surrounding the charged groups in side chains of poly(L-lysine) is gradually destroyed with a decreases in w_0 value, and thereby the electrostatic interaction between the cationic group of side chain and the anionic group of AOT molecule is enhanced. It is likely that the β -structure is more favorable than the α -helix structure, since AOT molecules are highly orientated along the polymer chain in the reversed micelles. Furthermore, the β -structure formed in the reversed micelles would be stable, because the hydrophobic chains of AOT molecules interacted with the side chains of polymer are faced to the bulk phase.

The conformation of poly(L-ornithine) was also studied in aqueous solutions containing various

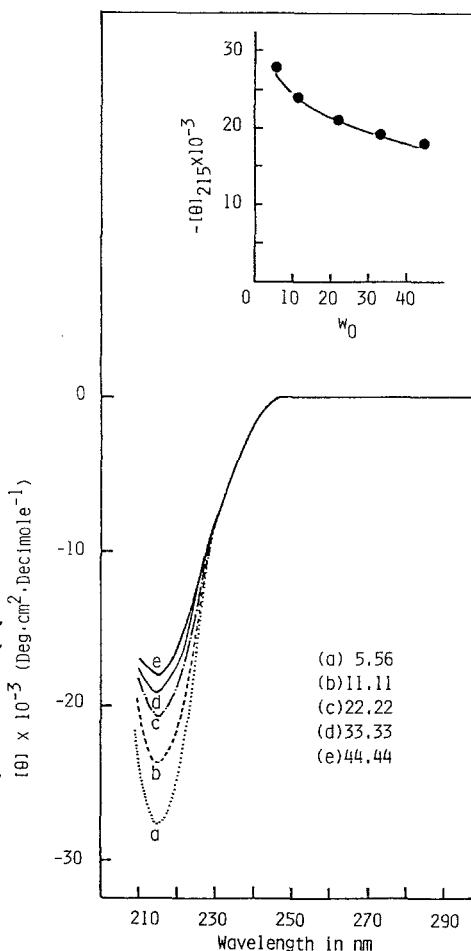


Fig. 4. CD spectra of poly(L-lysHBr) in reversed micellar solutions at different w_0 values

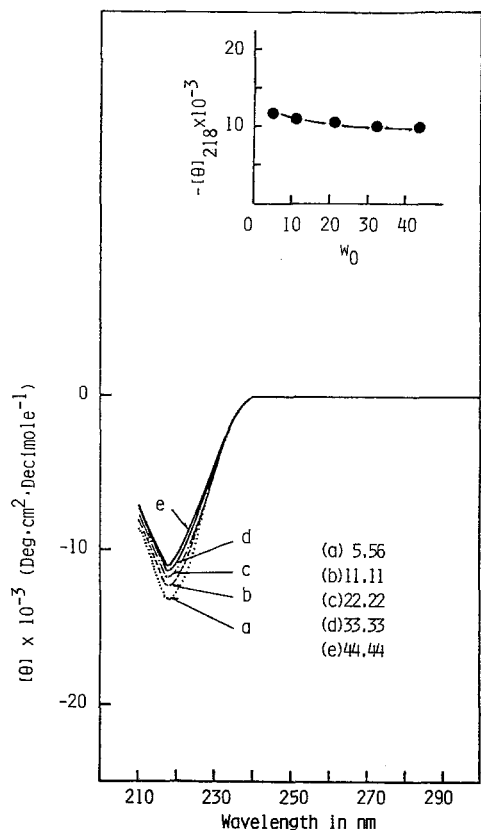


Fig. 5. CD spectra of poly(L-ornHCl) in reversed micellar solutions at different w_0 values

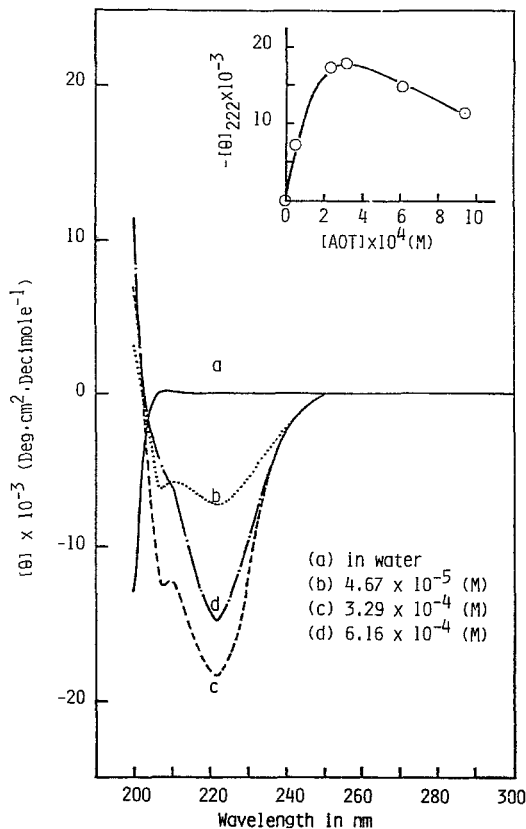
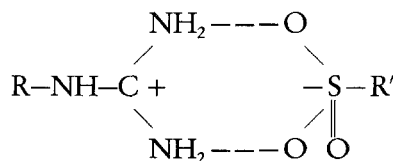


Fig. 6. CD spectra of poly(L-argHCl) in water and AOT solutions at $pH=6.2 \pm 0.2$

amounts of AOT. The conformational transition into an ordered structure occurs with the addition of AOT similarly to the case of poly(L-lysine), but the formation of the ordered structure was not so perfect. On the hand, the helix-inducing effect was not observed on other addition of sodium ethanesulfonate. Therefore, the ordered structure of poly(L-ornithine) seems to be induced by the electrostatic and hydrophobic interactions, similar to the case of poly(L-lysine). Figure 5 shows CD spectra of poly(L-ornithine) in the reversed micelles at various w_0 values, which reflect the formation of β -structure. The structure formation is somewhat enhanced as the w_0 value becomes smaller, but is not so extensive, compared with the case of poly(L-lysine).

Figure 6. shows CD spectra of poly(L-arginine) in aqueous solutions containing AOT. The addition of AOT into the aqueous solution of poly(L-arginine) leads to the conformational transition from coil to helix, followed by a superposition of α -helix and β -

structure. The helix formation is not observed in aqueous solutions containing sodium ethanesulfonate. It is suggested by these results that the α -helical conformation of poly(L-arginine) is formed as a result of the contributions of electrostatic and hydrophobic interactions, similar to the cases of poly(L-lysine) and poly(L-ornithine). It should be noted here that the interaction between the cationic group of the side chain of poly(L-arginine) and the anionic group of AOT may be effectively produced not only by the usual electrostatic interaction, but also by the hydrogen bond as shown in the following way, according to the suggestion of Zamma et al. [9], in the case of the interaction between the guanidinium group and the sulfate anion.



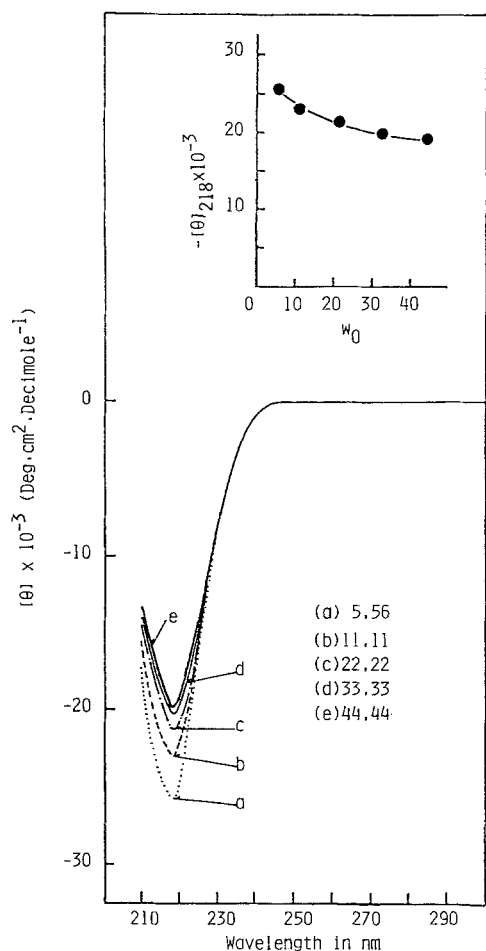


Fig. 7. CD spectra of poly(L-argHCl) in reversed micellar solutions at different w_0 values

The guanidinium group is considered to be the water structure breaking property [17]. Figure 7 shows CD spectra of poly(L-arginine) in the reversed micelles. The formation of β -structure is promoted as the w_0 value becomes smaller.

Figure 8 shows CD spectra of copoly(L-lys^{0.50}-L-leu^{0.50}) in aqueous solutions containing AOT. An ordered structure is promoted with an increasing amount of AOT, but further addition of AOT leads to a decrease in the magnitude of $[\theta]_{222}$, indicating the aggregation of polymer chains. These patterns of CD spectra seem to indicate the α -helical conformation mixed with a small ratio of β -structure. As is shown in Figure 9, it is difficult to decide whether this copolymer assumes an ordered structure in aqueous solutions containing sodium ethanesulfonate or not. Figure 10 shows CD spectra of the copolymer in the reversed micelles. In contrast to the cases of the homopolymers

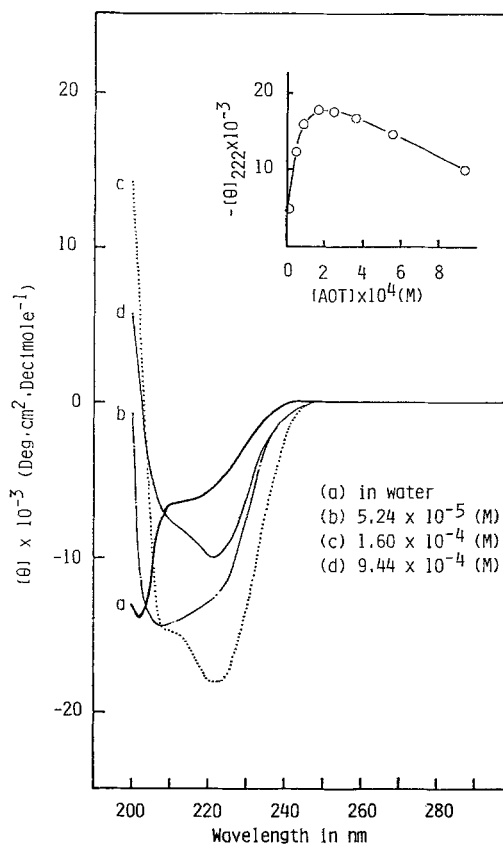


Fig. 8. CD spectra of copoly(L-lysHBr^{0.50}-L-leu^{0.50}) in water and AOT solutions at pH = 5.7 ± 0.2

such as poly(L-lysine), poly(L-ornithine), and poly(L-arginine), the rather shallow minimum at 222 nm decreases with decreasing w_0 value. This probably results from the fact that the hydrophobic interaction among leucine residues, which is responsible for the ordered structure, is weakened by a decrease of water molecules in the reversed micelles. This effect would be interpreted from the consideration that the hydrophobic hydration around the leucine residues is eliminated more extensively as the ratio of free water in the reversed micelles decreases.

Figure 11 shows CD spectra of copoly(L-lys^{0.37}-L-leu^{0.63}) in aqueous solutions containing AOT. This copolymer is able to take α -helix even in pure water, because the content of leucine residue is enough high to stabilize the α -helical conformation. Therefore, the addition of AOT to the aqueous solution leads only to the aggregation of α -helices. Figure 12 shows CD spectra of copoly(L-lys^{0.37}-L-leu^{0.63}) in the reversed micelles. The α -helix conformation of this copolymer is strongly depressed in the reversed micelles, suggest-

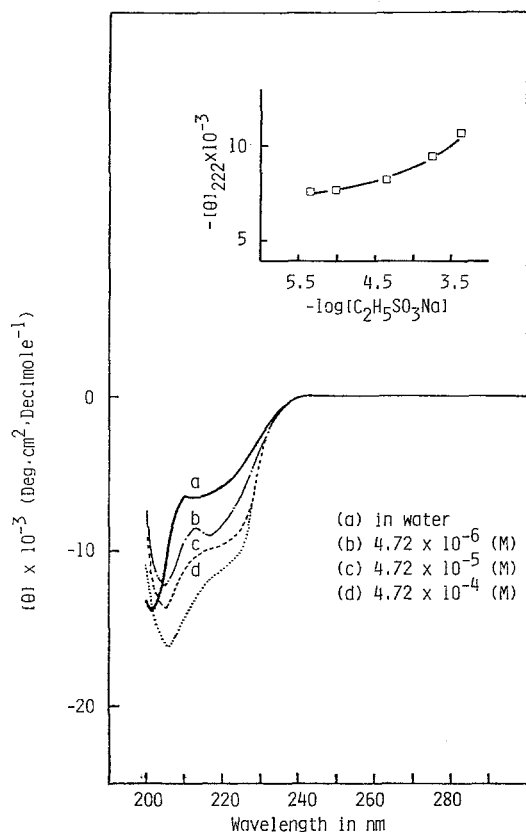


Fig. 9. CD spectra of copoly(L-lysHBr^{0.5}-L-leu^{0.50}) in water and sodium ethanesulfonate solutions at pH=6.0 ± 0.1

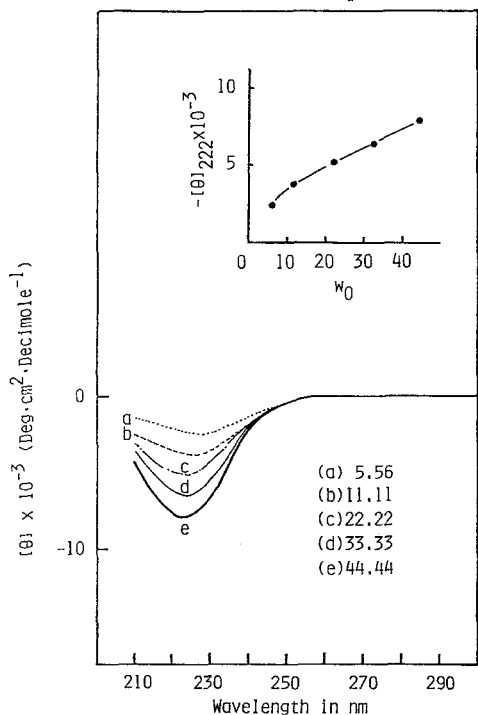


Fig. 10. CD spectra of copoly(L-lysHBr^{0.50}-L-leu^{0.50}) in reversed micellar solutions at different w_0 values

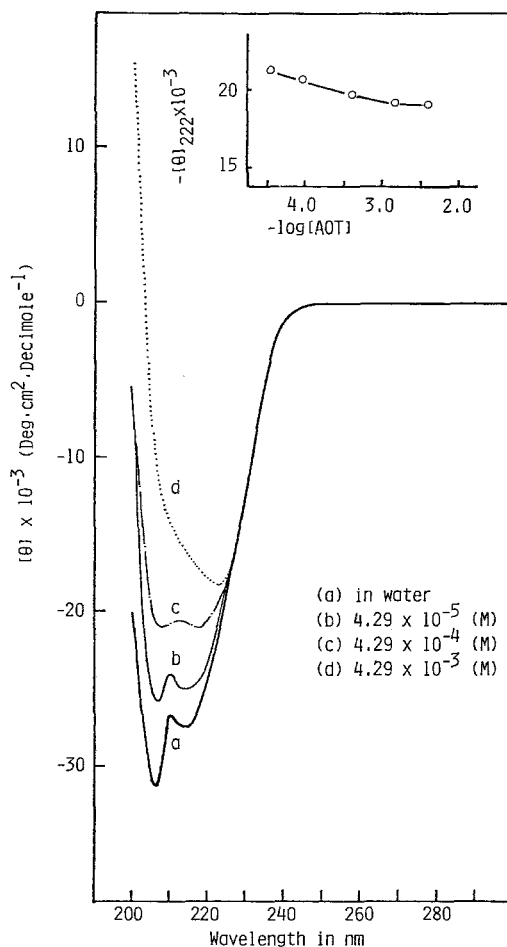


Fig. 11. CD spectra of copoly(L-lysHBr^{0.37}-L-leu^{0.63}) in water and AOT solutions at pH=5.9 ± 0.2

ing that the hydrophobic interaction among the leucine residues is effectively weakened in the reversed micelles. If the copolymer chain were partially exposed to the bulk phase of octane, the α -helix of copoly(L-lys^{0.37}-L-leu^{0.63}) might be fully maintained. It is reasonable, therefore, to consider from these results that the polymer chain is effectively entrapped in the reversed micelles.

Conclusion

The formation of an ordered structure of basic homopoly(α -amino acid)s such as poly(L-lysine), poly(L-ornithine), and poly(L-arginine) was effectively induced by the addition of AOT into the aqueous solution, but not induced by the addition of sodium ethanesulfonate. Such an inducing effect of ordered structure may be mainly due to the hydrophobic inter-

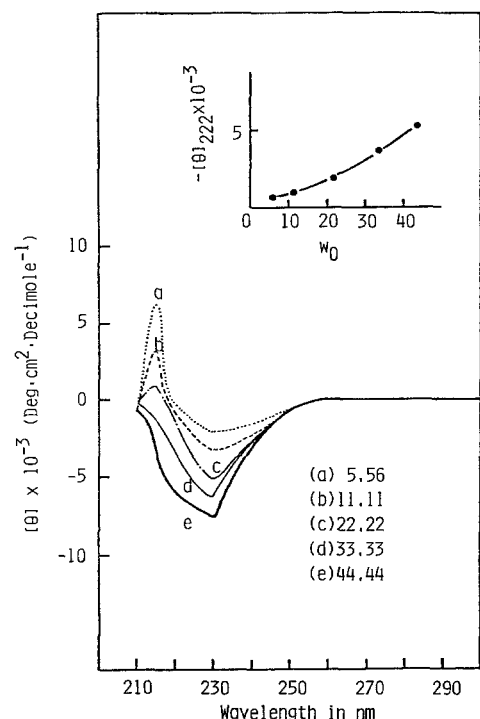


Fig. 12. CD spectra of copoly(L-lysHBr^{0.37}-L-leu^{0.63}) in reversed micellar solutions at different w_0 values

action between the methylene chains of AOT molecules orientated along the polymer chain. The electrostatic interaction between the cationic group of the polymer side chain and the anionic group of AOT is not enough to reduce the electrostatic repulsion of the polymer side chains, but seems to play a very important role for the orientation of AOT molecules along the polymer chain. In the reversed micelles, the β -structure is induced at low w_0 values. In this case, the electrostatic interaction between the cationic group in the side chain of polymer and the anionic group of AOT molecule became stronger as the amount of water decreased in the reversed micelle.

In contrast to these findings, copoly(L-lysyl-L-leucine) with a high content of leucyl residue does not assume a helical conformation in the reversed micelles. In this case the strong hydrophobic interaction among the leucine residues which could promote α -helix in pure water is weakened by the decrease of free water in the reversed micelle. Because this copolymer does not allow any helical conformation in the reversed micelles at low w_0 value, it could be reasonably said that the polymer chain is effectively entrapped in the reversed micelle.

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Received February 17, 1984;
accepted March 26, 1984

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