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The Conformational Changes of Poly(L-ornithine) with Organic Counter Ions in Aqueous Sodium 1-Decanesulfonate Solution

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Synopsis. The circular dichroism study was made of the coil-helix transitions of poly(L-ornithine) with 1-alkanesulfonate counter ions of the chain length from 3 to 6 in sodium 1-decanesulfonate solution. The conformational change is insensitive to the counter ion chain length and becomes appreciable even at surfactant concentrations as low as (1—2)$\times 10^{-4}$ mol dm^{-3}.

It is well known that additions of the ionic surfactants to the oppositely charged polypeptides in neutral solutions induce conformational transitions from coil to ordered structures.[1-9] For instance, poly(L-ornithine) (PO) undergoes a coil to helix transition in solutions of anionic surfactants such as sodium alkyl sulfate[10-12] and alkanesulfonate[8,9] even at remarkably lower concentrations than critical micelle concentrations. It has been inferred from the binding isotherm measurements that the surfactant ion binds cooperatively onto PO chain to give rise to a micelle like cluster.[8,9,12] The presence of such clusters has been pointed out to be essential for the surfactant-induced coil to helix transition of PO.[8]

So far as we know, however, all of the studies reported so far are concerned only with the ionic polypeptides with inorganic counter ions. It is thus interesting to study the surfactant induced conformational change of polypeptide with organic counter ion of varying hydrophobicity, since the presence of such counter ions is anticipated to affect the binding characteristics of surfactant ion. In this paper we report on the conformational changes of poly(L-ornithine) with 1-alkanesulfonate counter ions of carbon atoms n=3, 4, 5, and 6 (PO·C$_n$) in sodium 1-decanesulfonate (SDeS) solution.

Experimental

SDeS was synthesized from vacuum distilled 1-bromodecane by the sulfonation with sodium sulfite. Sodium 1-alkanesulfonates (Guaranteed grade, Tokyo Kasei Kogyo Co., Ltd.) were used without further purifications. Hydrochloride of PO (PO·Cl) and PO·C$_n$ were prepared by dialyzing hydrobromide of PO (Sigma, MW=41000 for n=5 and 6, and 25000 for the rest) against 0.1 mol dm^{-3} HCl and the corresponding sodium 1-alkanesulfonate respectively, and then against distilled water. The polypeptide concentration (C_p) in molarity of ionic groups was determined by colloid titration with poly(potassium vinyl sulfate) (Wako Pure Chemical Industries Ltd.).

Circular dichroism (CD) spectra of PO·Cl and PO·C$_n$ were measured as a function of SDeS concentration under the condition of constant C_p of 2.5×10^{-4} mol dm^{-3}. The spectra were recorded on JASCO spectropolarimeter J-20A. The potentiometric measurement on PO·C$_5$ - SDeS system was made with a cell based on nitrobenzene membrane electrode. The construction of the cell was described elsewhere.[13] All measurements were conducted in the absence of added salt at 25 °C.

Results and Discussion

Figure 1 shows the typical CD spectra of PO·C$_5$ recorded shortly after mixing with SDeS solutions of varying concentrations (C). Similar results are also obtained for PO·Cl and PO·C$_n$ with n=3, 4, and 6. In all cases, the CD spectrum varies successively from a characteristic spectrum of random coil at C=0 to that of helical conformation with a double minimum located around 207 and 222 nm. The presence of the isoelliptic point at 205 nm suggests the occurrence of a simple conformational transition from coil to helix under our experimental conditions.

According to the theory of polyelectrolyte solutions, the fraction of condensed counter ions is given by

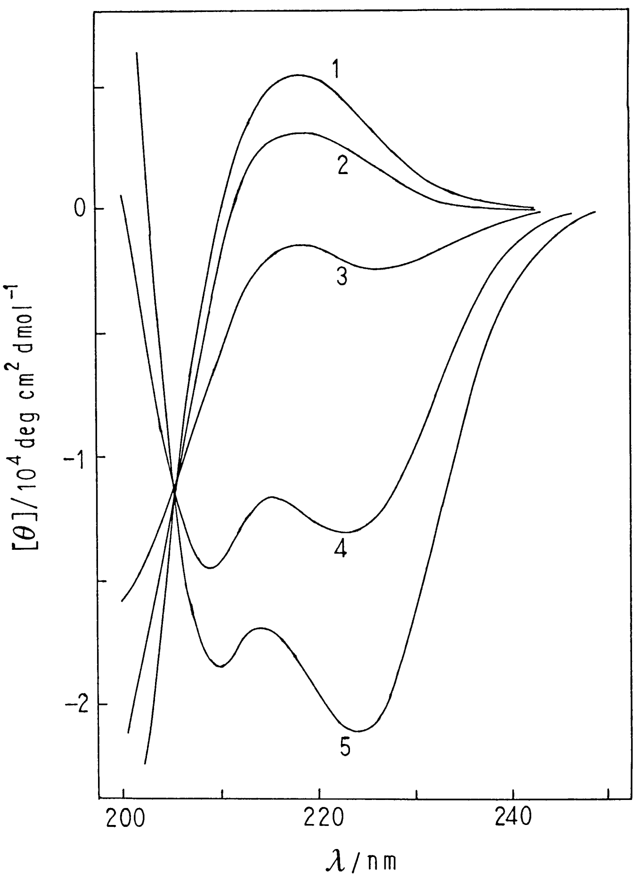

Fig. 1. The change in CD spectrum of PO·C$_5$ with SDeS concentration (C). C_p=2.5$\times 10^{-4}$ mol dm^{-3}; C/mmol dm^{-3}=0 (1), 0.25 (2), 0.5 (3), 1.25 (4), 3.0 (5).

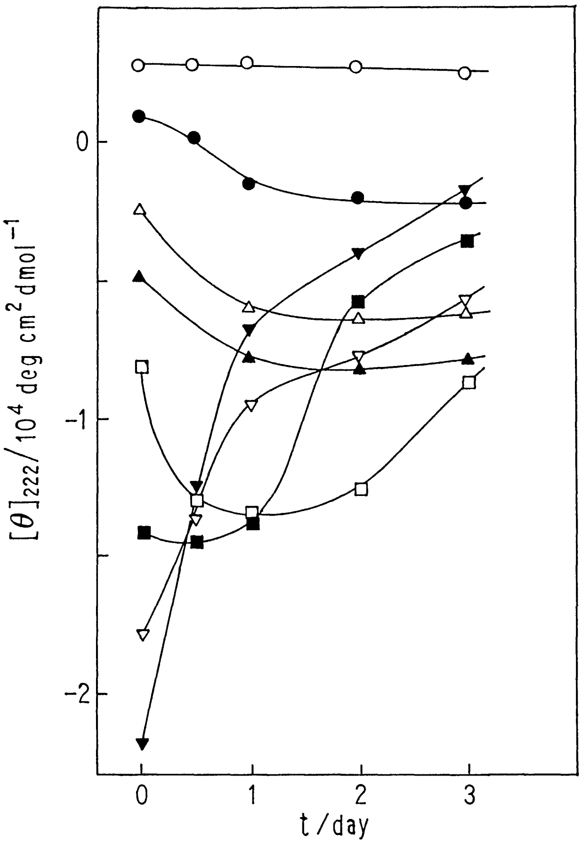

Fig. 2. The time-course of $[\theta]_{222}$ for PO·C_5 in SDeS solutions. $C_p=2.5\times10^{-4}$ mol dm^{-3}; C/mmol dm^{-3}=0 (○), 0.50 (●), 0.75 (△), 1.00 (▲), 1.25 (□), 1.50 (■), 2.00 (▽), 3.00 (▼).

$1-1/\xi$, where ξ represents a dimensionless structural parameter defined in terms of the average axial charge spacing (d) in nm as $\xi=0.714/d$ in water at 25 °C.[14,15) For the present systems, the structural consideration suggests that roughly 50% of counter ions are condensed onto PO chain. Therefore, it is highly probable that the average hydrophobicity around PO chain increases monotonically with increasing chain length of the counter ion even in the absence of surfactant ion. Nevertheless, CD spectrum of PO shows the characteristics of a random coil regardless of n. This fact implies that the presence of condensed hydrophobic counter ions with n up to 6 is insufficient to induce a conformational change of PO·C_n alone.

The CD spectrum of PO·C_n in SDeS solution varies gradually over a period of days on standing. A typical example for PO·C_5 is shown in Fig. 2. At lower surfactant concentrations, the mean residue ellipticity at 222 nm, $[\theta]_{222}$, the magnitude of which is proportional to the helical content, decreases monotonically with time and tends eventually to a constant. At higher surfactant concentrations, however, $[\theta]_{222}$ goes through a minimum or increases rapidly with time, because of the progress of aggregation process. Taking these into consideration, we adopted the minimum value of $[\theta]_{222}$ as a measure of helical content at a given surfactant concentration.

In Fig. 3 are plotted the values of $[\theta]_{222}$ thus determined as a function of surfactant concentration. In all cases, the helical content of PO increases monotonically with increasing surfactant concentration and becomes eventually constant at higher concentrations where the visible precipitate of PO—surfactant complex appears.

An interesting feature of Fig. 3 is an unexpected resemblance among $[\theta]_{222}$ vs. C curves for PO with counter ions of varying hydrophobicity. As a result of counter ion condensation phenomenon as noted above, the average hydrophobicity around binding

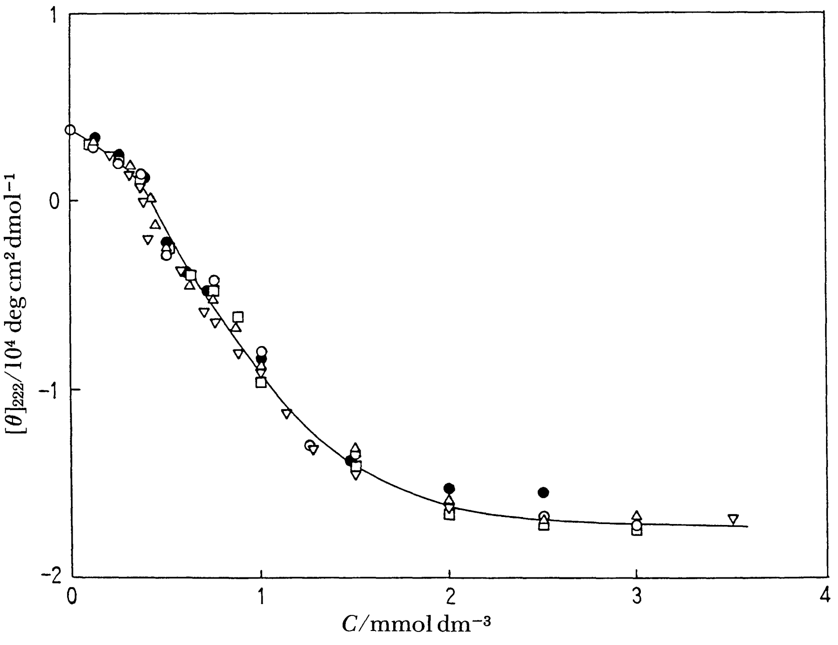

Fig. 3. The change in $[\theta]_{222}$ for PO·Cl and PO·C_n with SDeS concentration. $C_p=2.5\times10^{-4}$ mol dm^{-3}: ○, PO·Cl; △, n=3; □, n=4; ▽, n=5; ●, n=6.

site is supposed to increase regularly with increasing chain length of the counter ion. This effect will bring about a change in the intrinsic binding constant of the surfactant ion to PO. In addition, the bound surfactant ions are anticipated to form a mixed cluster with condensed counter ions, especially of a longer chain length. In solutions of PO·C_n, therefore, the binding mechanism on which the conformational change rests is considered to differ somewhat from that in solutions of PO with inorganic counter ions. In the latter case, the average cluster size of the bound surfactant ions is pointed out to be a primary determinant of the surfactant induced coil to helix transition.8) Although the detailed explanation for the observed resemblance among $[\theta]_{222}$ vs. C curves can not be offered at the present stage, it is probable that the overall hydrophobicity of PO·C_n-surfactant complex at a given surfactant concentration is nearly identical with one another regardless of the counter ion chain length.

Another feature of Fig. 3 is the onset of conformational change at remarkably low concentration range as low as (1—2)$\times 10^{-4}$ mol dm^{-3}. This behavior is in marked contrast with that of PO·Cl in SDeS solutions in the presence of added NaCl.8,9) Under the conditions of $C_p=2.15\times 10^{-4}$ mol dm^{-3} and [NaCl]$=2.1\times 10^{-2}$ mol dm^{-3}, $[\theta]_{222}$ of PO·Cl was found to decrease abruptly around $C=7\times 10^{-4}$ mol dm^{-3}, where the steep rise in binding isotherm occurs.8,9) Moreover, an addition of the salt has been noted to affect the binding characteristics of the surfactant ion to polyelectrolyte in such a way that the logarithm of the equilibrium surfactant concentration at half binding decreases linearly with the logarithm of the added salt concentration.16—18) Thus the present result can reasonably be attributed to an onset of surfactant ion binding in the concentration range much lower than that in salt solutions. In order to make this point clear, the potentiometric study was made for PO·C_5 system. Figure 4 shows the plots of electromotive force (E) of the cell vs. log C for SDeS solutions with and without PO·C_5. In the absence of PO·C_5, the electrode responds to the 1-decanesulfonate ion down to 5×10^{-5} mol dm^{-3} with a slope of 58.2 mV. In the presence of PO·C_5, E vs. log C curve is seen to deviate remarkably from that of SDeS solution alone. Under our experimental conditions, the interference of 1-pentanesulfonate ion can safely be neglected since the selectivity coefficient of 1-pentanesulfonate ion with respect to 1-decanesulfonate ion is of the order of 3×10^{-3}.19) Unfortunately, we can not construct the binding isotherm from Fig. 4 because of the lack of a detailed knowledge of the counter ion activity coefficient in such a complex system as surfactant-polyelectrolyte solution without added salt. With these reservations in mind, an equilibrium surfactant concentration is considered to be small enough even at the lowest concentration studied ($C=2\times 10^{-4}$ mol dm^{-3}). This in turn supports the foregoing presumption that the surfactant ion binding starts at remarkably low concentration range around (1—2)$\times 10^{-4}$ mol dm^{-3}.

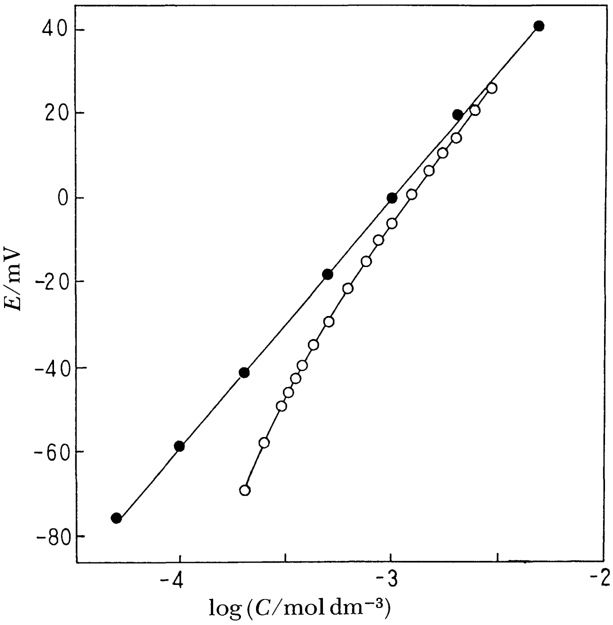

Fig. 4. Plots of the electromotive force (E) of the cell vs. log (C/mol dm^{-3}).
●, SDeS alone; ○, in the presence of PO·C_5 ($C_p=2.5\times 10^{-4}$ mol dm^{-3}).

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