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THE STEPWISE CONFORMATIONAL CHANGE OF POLY(L-LYSINE) IN AQUEOUS SOLUTION OF SODIUM 1-OCTANESULFONATE

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The circular dichroism spectrum of poly(L-lysine)(PLL) in aqueous 1-octanesulfonate solution was measured as a function of surfactant concentration at varying temperatures and the conformational phase diagram was constructed. At temperatures above 10°C, the increase in surfactant concentration causes PLL to change stepwise from coil to β-form and from β-form to helical conformation. Upon lowering temperature, the surfactant concentration range, where PLL assumes β-form, becomes narrower and eventually disappears at around 10°C.

An attention has recently been focused on the complicating features of the conformational transitions of poly(L-lysine)(PLL) homologs with side chain $R = -(CH_2)_nNH_2$ in anionic surfactant solutions.[1-10] With the exception of PLL($n = 4$), all other homologs ($n = 1 - 3$, $5 - 7$) are found to adopt a helical conformation in neutral solutions of sodium n-alkylsulfates.[1-5] In contrast, however, the conformational change of PLL in surfactant solution depends not only on the alkyl chain length of bound surfactant ion[2] but on solution pH.[6-8] Thus, PLL assumes β-form in solutions of sodium decylsulfate and the higher alkylsulfates,[2,3,6-10] but exceptionally a helical conformation in sodium octylsulfate solution[2] at neutral pH. In alkaline solution of sodium dodecylsulfate(SDS), PLL undergoes a reversible β-form to helix transition.[8]

These observations prompted us to study an unusual conformational change of PLL in solution of anionic surfactant with shorter alkyl chain length. In this communication is described an interesting finding that the conformational transition of PLL in 1-octanesulfonate solution takes place in two distinct steps, one from coil to β-form and the other from β-form to helix, with a change in surfactant concentration even at neutral pH.

PLL·HBr (Miles Laboratories Inc., M.W. = 37000) was converted to the corresponding hydrochloride through dialysis against 0.1 mol dm^{-3}(M) HCl and then distilled water. The residue concentration of PLL was determined by micro-Kjeldahl nitrogen analysis. Sodium 1-octanesulfonate of guaranteed grade (Tokyo Kasei Kogyo Co. Ltd.) was further extracted with petroleum ether and recrystallized twice from ethanol. The circular dichroism (CD) spectra were measured with a Jasco J-20A Spectropolarimeter under constant nitrogen flush, using a cell equipped with water-circulating jacket. The measurements were carried out at 5, 10, 20, 35, and 45 °C under the fixed concentrations of PLL (2.11×10^{-4} residue M) and NaCl (2.15×10^{-2}M).

In Fig. 1 is shown the typical change in CD spectrum of PLL with respect to surfactant concentration at 20 °C. PLL appears to lack any ordered structure in dilute surfactant concentration range below 5.5×10^{-3} M, since the presence of surfactant ion does not affect appreciably the CD spectrum characteristic of PLL in its random conformation.[11)] However, in narrow concentration range between 5.5×10^{-3} and 6.3×10^{-3} M, the CD spectrum varies abruptly with surfactant concentration to a spectrum which has single minimum centered at 218 nm with mean residue ellipticity, $[\theta]_{218}$, of -9000 deg cm^2 dmol^{-1}. A similar change in CD spectrum has already been observed for coil - β-form transition of PLL in sodium alkylsulfate solutions.[2)] For example with sodium decylsulfate, the CD spectrum of PLL shows a minimum located at 219 nm with $[\theta]_{219}$ of -11000 and a crossover point at 208 nm.[2)] A striking resemblance of the present CD spectral change to that in sodium decylsulfate solution suggests that PLL also undergoes a cooperative transition from random coil to β-form in this narrow concentration range. The shape and magnitude of the CD spectrum remains virtually constant and PLL retains the β-form up to 7.5×10^{-3} M. An anomalous behavior of PLL in 1-octanesulfonate solution is an appearance of second transition region between 7.5×10^{-3} and 8.7×10^{-3} M, where the CD spectrum changes drastically to the characteristic spectrum of α-helix. The resulting CD profile resembles that of helical poly(L-ornithine) in SDS solution[2)] in displaying a double minimum located at 208 and 222 nm with residue ellipticity of -26000 and -30000, respectively. The double minimum feature persists up to the

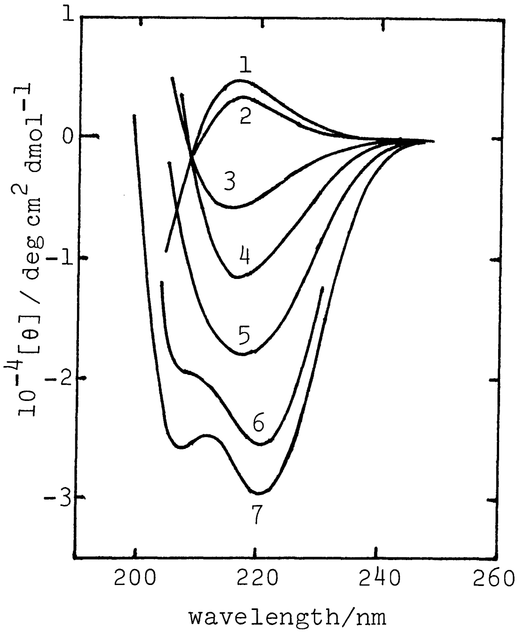

Fig. 1. The CD spectra of PLL in sodium 1-octanesulfonate solutions at 20 °C.
PLL; 2.11×10^{-4} residue M: NaCl; 2.13 $\times 10^{-2}$ M: 10^3[1-octanesulfonate]/M; 1, 0.0; 2, 5.57; 3, 6.07; 4, 7.79; 5, 8.10, 6, 8.30; 7, 10.1.

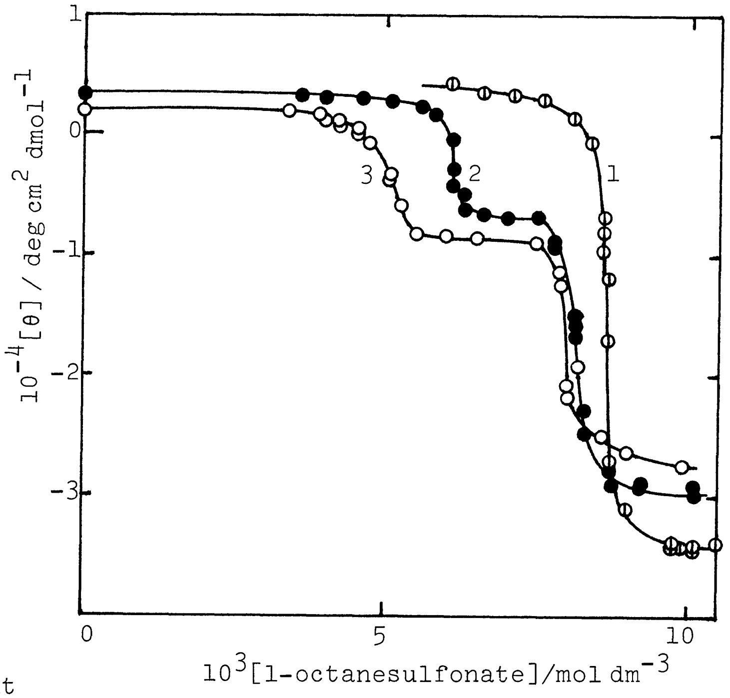

Fig. 2. The plots of $[\theta]_{222}$ versus the concentration of 1-octanesulfonate.
1, 5 °C; 2, 20 °C; 3, 45 °C.

surfactant concentration at which the precipitation of PLL - surfactant complex occurs. Near the critical concentration for precipitation at around 1.2×10^{-2} M, the solution becomes slightly turbid and the CD minimum at 210 nm changes rapidly with time into shoulder, presumably by virtue of gradual growth of the precipitate.

In order to show more clearly the stepwise transition of PLL in 1-octanesulfonate solution, the residue ellipticity at 222 nm, which is a measure of helix content in case of helical conformation, is plotted in Fig. 2 against surfactant concentration at varying temperatures. It can immediately be seen that the conformational transition of PLL takes place cooperatively in two distinct steps at higher temperatures; a plateau at intermediate concentration range represents the region of β-form, while that at higher concentration denotes a helical conformation. The CD of β-form PLL tends to increase in magnitude successively with raising temperature. In fact, the $[\theta]_{218}$, which is proportional to the β-form content, changes from -8100 at 10 °C to -11200 at 45 °C, suggesting the stabilization of surfactant induced β-form by heating. The progressive broadening in the β-form region with raising temperature would provide an additional evidence for the greater thermal stability of β-form PLL at elevated temperatures. On the other hand, the opposite trend is observed with $[\theta]_{222}$ of helical PLL. Satake and Yang[2)] have ascribed a similar change in $[\theta]_{222}$ for helical poly(L-ornithine) in SDS solution to a partial breaking up of the helix at elevated temperatures. This will also be true for the present system. It seems, thus, highly probable that the surfactant induced ordered structures contain a small amount of random portion depending on temperature.

With these considerations in mind, we can construct a conformational phase diagram of PLL in 1-octanesulfonate solution. Figure 3 shows such a diagram constructed from the data of Fig. 2 together with those at 10 and 35 °C. The transition regions are specified by the shaded area, since the conformational change of PLL occurs actually over a narrow but definite concentration range of the surfactant. The coil - β-form transition concentration is seen to increase successively with lowering temperature. On the other hand, the β-form - helix transition concentration shows similar but much less marked temperature dependence. As a result, they intersect each other at around 10 °C, below which the β-form region is no longer detectable. It is to be emphasized, however, that the disappearance of β-form region does not necessarily imply the direct transition of PLL from coil to helical conformation below this temperature. It is still possible that PLL undergoes a

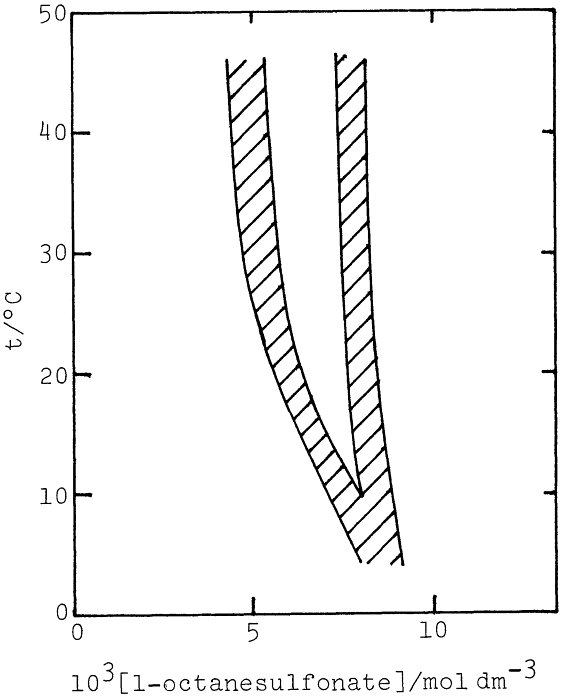

Fig. 3. The conformational phase diagram of PLL in sodium 1-octanesulfonate.

transition from coil to helical conformation through a β-form within quite narrow transition region. This is indeed proved to be the case. Even at 5 °C, PLL shows the CD spectrum indicative of the presence of β-form at intermediate concentrations in the transition region depicted in Fig. 3. As previously noted, the region of helical PLL extends to the surfactant concentration at which PLL-surfactant complex precipitates.

In their study of the conformational transition of PLL in alkaline solution of SDS, Satake and Yang8) found that PLL undergoes reversible transition from β-form to helix upon raising solution pH to, e.g., 11.3 - 11.9 at 20 °C. Similar pH induced β-form - helix transition of PLL in SDS solution was also studied by Feldshtein et al.6) and Zezin et al.7) In these cases, the decrease in the degree of binding of surfactant ion arising from the deprotonation of side chain amino group of lysyl residue plays an important role in the β-form - helix transition of PLL.6-8) Since under our experimental conditions, however, the solution pH is kept neutral throughout whole experiments, the deprotonation effect should necessarily be ruled out. In another experiments with sodium n-alkanesulfonates with 10, 11 and 12 carbon atoms, we found that the increase in surfactant concentration causes PLL to change only from coil to β-form regardless of temperature. The only difference between 1-octanesulfonate and its higher homologs lies in hydrophobic alkyl chain length, reflecting the importance of hydrophobic interaction between bound surfactant ions in determining the conformation of polypeptide in surfactant solution. However, because of the lack of the detailed information about the factors which influence the type of surfactant induced polypeptide conformation, the mechanism of the stepwise conformational change of PLL in 1-octanesulfonate solution remains unclear at the present stage.

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