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Kinetic Study on Conformational Changes of Poly(L-lysine) in Sodium Alkyl Sulfate Solutions. Effects of Surfactant Chain Length and Added NaCl

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The conformational changes of poly(L-lysine) (PLL) were observed in alkyl sulfate solutions by a circular dichroic method. PLL forms a β-structure in sodium dodecyl sulfate solution with an apparent first-order rate constant of 3.0×10^{-2} s^{-1} (20 °C). In sodium octyl sulfate (SOS) solution, PLL assumes the α-helix within a certain concentration range of the surfactant, above which the β-structure is favored. The rate of coil to β-structure transition slows down with increase in hydrophobic interaction between surfactants and PLL. The presence of sodium ions decreases the rate of coil to α-helix transition by about two orders of magnitude (2.0×10^{-1} s^{-1} salt free to 3.0×10^{-3} s^{-1} in the presence of 2.0×10^{-2} mol/dm^3 NaCl at 20 °C). These phenomena are explained in terms of a rearrangement process of bound surfactants adjusting for a free energy minimum; a stronger binding force brings about a slower rearrangement rate.

Extensive circular dichroism (CD) studies have been made on the conformational changes of polypeptides in surfactant solutions. Poly(L-lysine) (PLL) has been an interesting material, since it can take on three kinds of conformations depending on experimental conditions.[1-8] For example, the PLL conformation is changed to β-structure in the presence of sodium dodecyl sulfate (SDS) at neutral pH.[1,3,6] However, few kinetic aspects of the conformational changes of PLL in surfactant solutions have been studied. We have applied a CD stopped-flow method to the study of the conformational changes of the PLL in sodium octyl sulfate (SOS) solution.[9] We reported the SOS concentration dependences of both the PLL conformations and the rate constants of the conformational changes; our results are briefly summarized as follows.[9] PLL adopts an α-helix only in a certain concentration range of SOS, taking a disordered structure below the SOS concentration range and β-structure above it. The rate constant of coil to β-structure transition sharply increases with SOS concentration, while that of coil to α-helix transition is almost independent of SOS concentration.

This work examined the above mentioned dependences in the presence of NaCl and also in solutions of an alkyl sulfate with different chain lengths. The main purpose of this paper is to discuss the effects of the chain length of alkyl sulfate and the added NaCl on the conformational changes.

Experimental

The sources of PLL and SOS have been described elsewhere.[9] The SDS is the same sample as before.[10]

The measurements of CD were carried out with a JASCO J-500A spectropolarimeter equipped with a JASCO DP-501 data processor, using a 1.0 mm path length cell with water-circulating jacket. The path length of a cell used in a stopped-flow measurement was also 1.0 mm.[9] Details of the CD and the stopped-flow measurements have been stated previously.[9]

Results

The addition of SDS causes a conformational change of PLL from coil to β-structure, as seen in Fig. 1; this

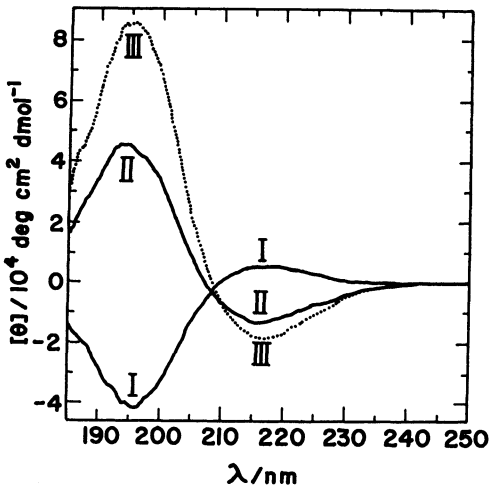

Fig. 1. Typical CD spectra of PLL solutions in the absence (curve I) and the presence (curve II) of 10 mM SDS and the difference CD spectrum between them (curve III) at 20 °C. The concentration of PLL was 2.8×10^{-4} M (residue). The time constant and the scanning speed of the spectropolarimeter were 1.0 s and 20 nm/min, respectively. These spectra are averaged over 8 repetitions and the difference CD spectrum was directly obtained using the data processor.

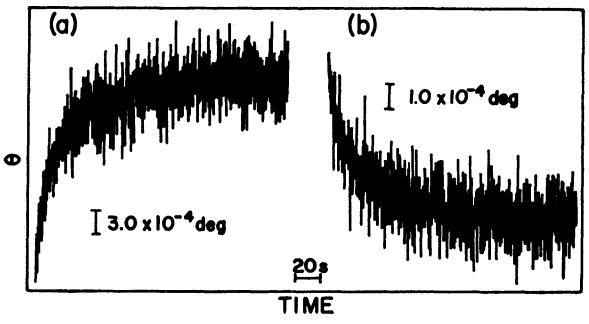

Fig. 2. Typical time courses of ellipticity changes at 195 (a) and 217 nm (b) at 20 °C. The traces (a) and (b) are averages of 8 and 2 repetitions, respectively. The final concentrations of PLL and SDS were 2.8×10^{-4} M (residue) and 10 mM, respectively.

result was previously reported by other investigators.[1,3,6] The CD stopped-flow measurements on the conformational change were made by tracing ellipticities at 195 and 217 nm, where positive and negative peaks appeared in the difference CD spectrum (III in Fig. 1). Typical time courses of the ellipticity changes at both wavelengths are seen in Fig. 2. The direction of the ellipticity change at each wavelength is in accord with the results in Fig. 1, suggesting that the observed process is due to the conformational change from coil to β-structure. The conformational change obeyed first-order kinetics in the ordinary treatment.[9] The rate constant was $3.0\times10^{-2}\,s^{-1}$ (20 °C), and was independent of the SDS concentration (not shown here).

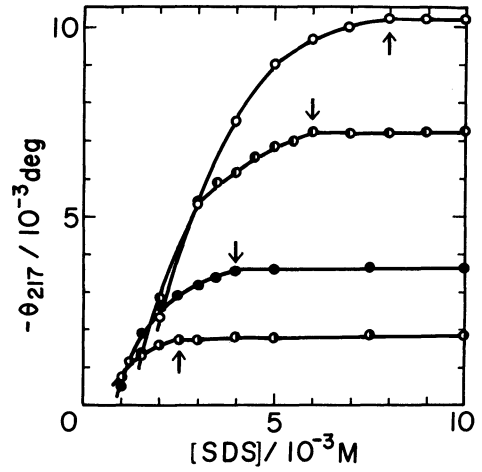

Fig. 3. Effect of PLL concentration on ellipticity, θ_{217} at various SDS concentrations. Residual PLL concentrations are 1.4 (◑), 2.8 (●), 5.6 (◐), and 7.9×10^{-4} M (○). These data were obtained from the measurements under the same experimental conditions as in Fig. 1 except for PLL concentration.

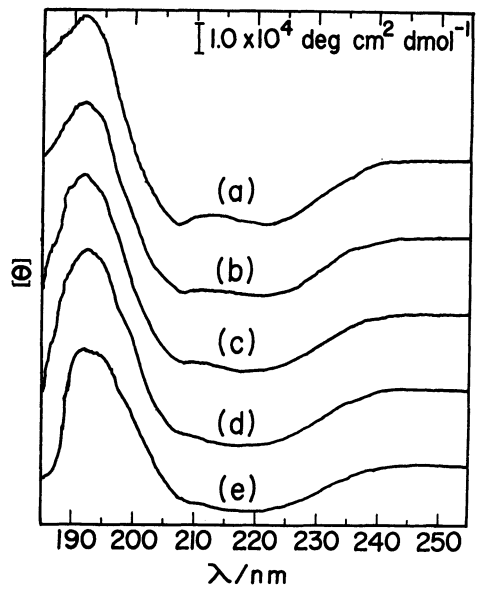

Fig. 4. Changes of CD spectrum of PLL in 5.0 mM SOS solution by addition of NaCl at 20 °C. NaCl concentrations: (a); 0, (b); 5.0, (c); 10.0, (d); 15, and (e); 20 mM.

Figure 3 shows the plots of the ellipticities at 217 nm, θ_{217} *vs.* SDS concentration at several PLL concentrations. The conformation of PLL grows with the SDS concentration after a sudden increase in ellipticity. The increase in PLL concentration shifts the range of the SDS concentration where the sudden conformational change of PLL occurs and eventually the conformational change is completed, as seen in Fig. 3.

Figure 4 shows that the CD spectrum of α-helical PLL in the SOS solution is not appreciably affected at a low NaCl concentration; the double negative maximum gradually fades out with increase of NaCl concentration. Since the NaCl added beyond 25 mM (the concentration unit: 1 M = 1 mol/dm^3 is used) decreased the ellipticities over the entire wavelength range, the effects of the added salt were examined in the concentration range below 20 mM. The addition of NaCl does not change the characteristic concentration ranges of the surfactants where the conformation of PLL changes, although the critical micelle concentration sharply changes on addition of such an electrolyte. The added NaCl has some effect on the rate constants of the conformational changes in SOS solution and their SOS concentration dependences, as depicted in Figs. 5 and 6. Figure 5 shows the increase of the rate constant with SOS concentration in the presence of NaCl. This exhibits marked contrast with the insensitiveness to SOS concentration in such a concentration range without NaCl.[9] The combination of the 4 figures in Fig. 5 also indicates that the rate constant, k, decreases with increase in the added NaCl concentration at each SOS concentration. As seen in Fig. 6, the presence of NaCl markedly decreases the rate constant of the conformational change at 4.0 mM SOS, which is the lowest concentration where a coil to α-helix transition appears.[9] The rate constant rapidly decreased up to 10 mM NaCl and then gradually leveled off. On the

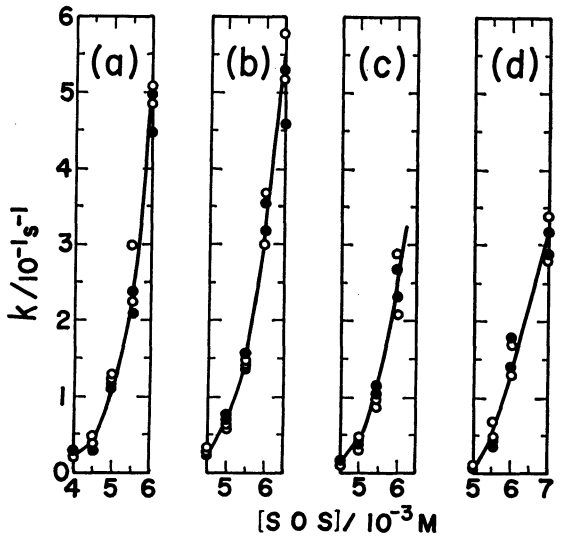

Fig. 5. SOS concentration dependences of rate constant, k, in the presence of NaCl at 20 °C. NaCl concentrations: (a); 5.0, (b); 10.0, (c); 15, and (d); 20 mM. The rate constants were obtained from ellipticity changes at 192 (○) and 221 nm (●) with time. PLL concentration was 2.8×10^{-4} M (residue).

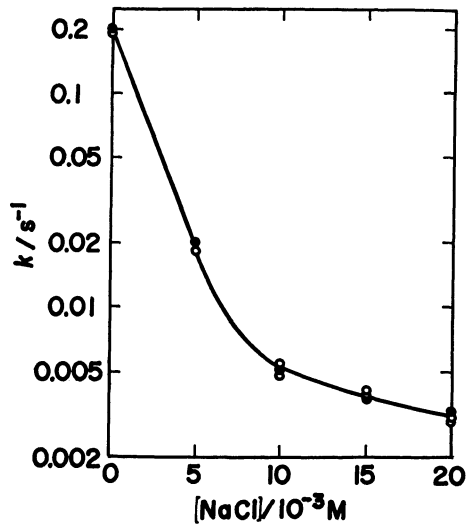

Fig. 6. Effect of NaCl on rate constant of coil to α-helix transition in 4.0 mM SOS solution at 20 °C. The solid and open circles respectively denote the same meanings as in Fig. 5. PLL concentration was 2.8×10^{-4} M (residue).

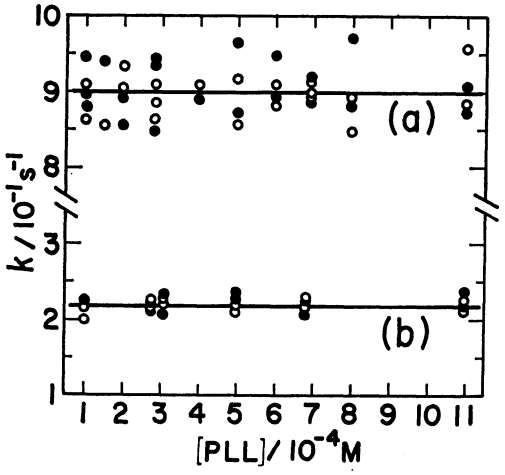

Fig. 7. PLL concentration dependences of rate constants of conformational changes of PLL in 5.0 mM (a) and 8.0 mM (b) SOS solutions at 20 °C. The solid and open circles denote the same meanings as in Fig. 5.

other hand, the presence of NaCl does not affect the rate constant of coil to β-structure transition in SDS solution.

The PLL concentration dependences of two kinds of rate constants, one for the coil to α-helix transition and the other for the coil to β-structure one, were examined in SOS solution, as shown in Fig. 7. The rate constant is almost constant (irrespective of the PLL concentration) in each case. In the same PLL concentration range, the rate constant of coil to β-structure transition induced by SDS is not affected by changing PLL concentration (not shown here).

Discussion

Effect of Chain Length of Alkyl Sulfate on the Conformational Change. As previously reported,[9] PLL adopts an α-helical state in a certain concentration range of SOS. In the PLL residual concentrations between 1.4—7.9×10^{-4} M, the characteristic SOS concentration range remains almost unchanged. The SOS concentration dependences of residual ellipticities at 192 and 221 nm, $[\theta]_{192}$ and $[\theta]_{221}$ do not differ from those in Fig. 2 of the previous paper.[9] On the contrary, the PLL adopts a β-structure in a solution of SDS whose concentration range is similar to that of SOS. The SDS concentration range where the coil to β-structure transition is observed is clearly dependent on PLL concentration, as seen in Fig. 3; this indicates that about 10 SDS molecules are required for 1 residue of PLL when the conversion to β-structure completes. It is expected that the binding ratio of surfactant to polypeptide determines the conformation of the surfactant-polypeptide complex. The fact that the effect of the PLL concentration on the range of the characteristic surfactant concentration is quite different for these two surfactants may indicate that the β-structured PLL binds a large number of surfactant molecules, while the α-helical PLL does not require so many bound surfactants. It is also expected that the ratio of bound surfactants to free ones is smaller in the case of SOS than in the other because of the difference in binding forces to the polypeptide. Therefore, the SOS concentration range where the coil to α-helix transition is induced is apparently independent of the PLL concentration. This is in line with the results by Satake and Yang[8] that PLL and poly(L-ornithine) (PLO) adopt β-structure and α-helix, respectively, in sodium decyl sulfate solution, and that such a difference is also reflected in the binding isotherm, which is much steeper for the β-structured polypeptide than the α-helical one. Alkyl sulfates may be probably oriented in the same direction on the β-structured polypeptide, while they may be oriented radially on the α-helical one. So, alkyl sulfates would be in contact with each other more efficiently on the β-structure than on the other, leading to a stronger binding. Such a difference in the mode of binding would reflect the difference in the amounts of surfactants bound to PLL.

The conformational change of PLL induced by SDS was followed as the ellipticity changes at 195 and 217 nm with time. The observed process must be the change from random coil to β-structure, on the basis of the comparison of the time courses in Fig. 2 with the changes of CD spectra in Fig. 1. The first-order rate constant of the conformational change of PLL induced by SDS is *ca.* 3.0×10^{-2} s^{-1} (20 °C); it is independent of SDS concentration. This rate constant is slower than that of the SOS-induced coil to β-structure transition ($6—10 \times 10^{-1}$ s^{-1}). The clear distinction in the transition rates must be attributed to the differences in the chain length of alkyl sulfates, since binding energies are different for these surfactants. It should be noted that the rate constants of the conformational changes are quite independent of PLL concentrations in SOS solution, as seen in Fig. 7. These facts would suggest that the binding process between the surfactants and the polypeptide is not a rate-determining step in the conformational changes. Alkyl sulfates bound to

polypeptides can be in contact with each other through their alkyl chains, and the conformational changes of the polypeptide must be made together with many surfactant molecules bound on it. In the course of a conformational change of polypeptide, some of the bound surfactants may be forced to move from the initial binding sites to some other sites to achieve a more thermodynamically favorable conformation. Through this process, the bulky hydrophobic groups of surfactants should find maximal contacts between the hydrophobic groups of bound surfactants and the hydrophobic parts of the polypeptide, reconciling with the structure formation to reach minimal free energy. In the initial binding stage before the conformational change, the interaction with SDS is stronger than that with SOS. So, the coil to β-structure transition in SDS solution seems to require a much longer time in the rearrangement process than that in SOS solution.

The rate constant for the coil to β-structure transition sharply depends on SOS concentration, as seen in Fig. 5 of Ref. 9, although it is independent of SDS concentration. The SOS concentration dependence of the rate constant may be due to the transitional concentration range of SOS where the coil to α-helix transition shifts to the coil to β-structure one. Subsequently, it might be expected that the rate of the coil to β-structure transition is independent of surfactant concentration except for a very narrow range of surfactant concentrarion where the cooperative conformational change begins to occur. This indicates that the transitional concentration range of SOS is wider than that of SDS because the cooperativity of conformational change is expected to be greater for SDS than for SOS.

Sarkar and Doty[1] and Li and Spector[3] suggest that the intramolecular β-structure of PLL is formed in SDS solution, but Satake throws some doubt on this.[11] If the intermolecular β-structure is formed, the rate of the coil to β-structure transition should be dependent on PLL concentration. However, the opposite is the case, as stated above, suggesting the probability of the intramolecular structure formation in both α-helix and β-structure. In addition, the coil to β-structure transition obeys first-order kinetics, as in the case of the coil to α-helix one.[9] If the intermolecular β-structure is formed, the kinetics of the coil to β-structure transition will not be of first-order but of mixed order, as previously discussed by Snell and Fasman.[12] All these results would be indicative of the intramolecular β-structure formation in the surfactant solution. Whether the β-structure formation is intramolecular or intermolecular is considered to be essentially responsible for the degree of polymerization of the PLL used.[3,6,13] The intramolecular β-structure seems to be predominant in the present PLL with polymerization degree of 140.[9]

Effect of Added NaCl on the Conformational Change. Addition of NaCl as much as 10 mM seems to cause very slight changes in the α-helical conformation of PLL in SOS solution, as judged from the CD spectra in Fig. 4. The effect of added NaCl is so weak that the α-helical conformation itself does not change to some other conformation, in marked contrast with the effect of added 1-octanol which causes another transition from α-helix to β-structure.[9] The addition of the salt does not alter the surfactant concentration range where the conformation changes, probably because the amount of bound surfactant molecules is not affected by the presence of the salt.

As seen in Figs. 5 and 6, however, the addition of NaCl causes a considerable effect on the rate constant of the coil to α-helix transition in the SOS solution, while that of the coil to β-structure transition in the SDS solution is not affected by the addition of NaCl. As discussed above, the surfactant molecules are considered to be implanted radially on an α-helical polypeptide and the amount of bound surfactants seems to be much smaller on the α-helical polypeptide than on the β-structured one. This indicates that, although the surfactant molecules bound to the polypeptide would be in contact with each other, they are less compact when bound on α-helical polypeptide than on the other one. Sodium ions, as counter ions, must condense around the surfactant-α-helical polypeptide complex and weaken the repulsive force between the surfactants, just as in the case of ordinary micelle formation. Thus, the presence of NaCl can be considered to cause stronger interactions between the surfactants and the α-helical polypeptide. It is expected in such situations that the more strongly bound surfactants have a slower rearrangement process on the α-helical PLL. This phenomenon would markedly appear as the drastic decrease of the rate constant at 4.0 mM SOS, which is the lowest concentration at which the coil to α-helix transition is induced, as seen in Fig. 6. On the contrary, many surfactant molecules can bind onto the β-structured polypeptide with their hydrocarbon chains oriented in the same direction with strong contacts. The interaction between the surfactants is so strong that the added NaCl can hardly change the rate of the rearrangement of surfactant molecules.

In conclusion, the random coiled conformation of PLL changes to β-structure in the presence of SDS. This is because SDS gives a more hydrophobic atmosphere to host polypeptide than SOS which induces the coil to α-helix transition within the limited concentration range. The intramolecular β-structure is considered to be formed in the PLL with the polymerization degree of 140. The rate of coil to β-structure transition has a tendency to become slower with increase in the hydrophobic interactions of surfactants. The rate of the conformational change is affected very little by the added NaCl, probably because of the strong interactions between the surfactant molecules bound to the β-structured polypeptide. On the other hand, the added NaCl appreciably decreases the rate of coil to α-helix. This would be due to the fact that the surfactants bound to the α-helical PLL are less compact, and so the α-helical-surfactant complex is much more influenced by the addition of sodium ions.

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