Conformational Change of Poly(L-lysine) by Sodium Octyl Sulfate as Studied by Stopped-flow Circular Dichroism Method

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Circular dichroism (CD) studies were made on the conformational changes of poly(L-lysine) (PLL) induced by sodium octyl sulfate (SOS). The conformation of PLL has a negative double maximum characterizing the α-helix in 4.0—6.0 × 10^{-3} mol/dm^3 SOS solutions. At a higher concentration of SOS, PLL takes the β-structure, the random coil remaining below it. These conformational changes were followed by measuring ellipticities at 192, 207, and 221 nm according to a CD stopped-flow method. The rate constant was *ca.* 2.7 × 10^{-1} s^{-1} for the coil-helix transition, the transition rate from coil to β-structure being high depending on SOS concentration. The β-structure was attained by adding a small amount of 1-octanol to the α-helical PLL in 5.0 × 10^{-3} mol/dm^3 SOS. The effect of the surfactant on the triphasic conformation changes is discussed in terms of hydrophobic environment.

Conformational changes of polypeptides and proteins have been extensively studied by circular dichroism (CD) measurements. Poly(L-lysine) (PLL) has been frequently selected as a typical substance because of its diversity in conformations.[1-3] Satake and Yang gave an evidence that PLL adopts β-structure in the presence of sodium alkyl sulfates which have longer hydrocarbon chains than 10 carbons. They obtained a distorted CD spectrum reminiscent of α-helix,[3] which should show a characteristic CD spectrum with a double negative maximum. It has not been clarified which conformation PLL takes in a SOS solution. This paper reports the SOS concentration dependence of PLL conformation as well as the kinetics of conformational changes studied by the CD stopped-flow method.

Experimental

The surfactant, SOS, was prepared from 1-octanol (purity: 99%, Aldrich Chemical Co.) by the method of Dreger *et al.*,[4] and recrystallized twice from 2-propanol and once from pure water. The critical micelle concentration of the surfactant was determined to be 148 mM (as concentration unit, 1 M= 1 mol/dm^3, is used) at 25 °C by means of electric conductivity in agreement with that reported.[5] The hydrobromide salt of PLL (degree of polymerization: 140, Sigma Chemical Co.) was converted into hydrochloride by dialysis against 0.1 M HCl and then water. The concentration of PLL was determined by colloid titration.[6,7]

Measurements of CD were carried out with a JASCO-J500A spectropolarimeter (Japan Spectroscopic Co.) equipped with a DP-501 data processor. The data processor was partially modified to follow rapid reactions. The sampling time required for 5 data points/nm on a chart in both X and Y directions was selected from 1.0 × 10^{-4} to 1.0 × 10^{-1} s for kinetic measurements. The stopped-flow measurements by CD detection were carried out with a rapid mixer (Union Giken Co.) and a specially designed observation cell whose lightpath length and incident area being 1.0 mm and 1.0 cm^2, respectively. The dead volume beyond the mixer was calculated to be 250 μl for the stopped-flow system. The stopped-flow system was driven by *ca.* 2.0 kg/cm^2 compressed air.

Results and Discussion

Figure 1 shows typical CD spectra of PLL in the absence and the presence of 5.0 mM SOS and the difference CD spectrum between them. The conformation of PLL in the SOS concentrations between 4.0 and 6.0 mM seems to be the α-helical structure characterized by a double negative maximum. Satake and Yang reported that the CD spectrum of poly(L-ornithine)(PLO)–sodium dodecyl sulfate (SDS) complex is characteristic of a helical conformation with a double negative maximum, but the PLO–SOS and PLL–SOS complexes display the CD spectrum with a negative maximum at 225 nm and a shoulder near 210 nm suggesting a helical conformation.[3] The CD spectrum of PLL–SOS complex closely resembles that of PLO-

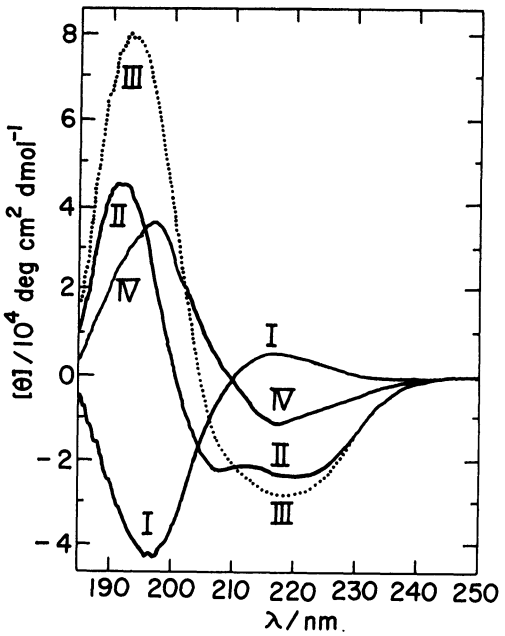

Fig. 1. Typical CD spectra of PLL solutions in the absence (curve I) and the presence (curve II) of 5.0 mM SOS and the difference CD spectrum between them (curve III) at 20 °C. Curve IV represents the CD spectrum of PLL in 7.5 mM SOS solution. The concentration of PLL was 2.8 × 10^{-4} M. The thickness of cell used was 1.0 mm. The time constant and the scanning speed of spectropolarimeter were 1.0 s and 20 nm/min, respectively. These spectra are averaged over 8 repetitions.

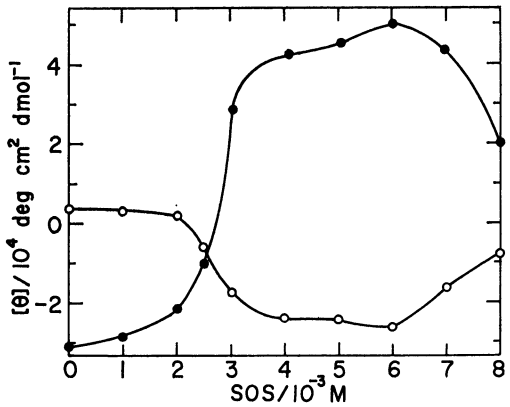

Fig. 2. Plots of $[\theta]_{192}$ (●) and $[\theta]_{221}$ (○) *vs.* concentration of SOS at 20 °C. These data were obtained from the measurements under the same experimental conditions as stated in Fig. 1.

SDS complex in their work. The magnitude of ellipticity of the PLL–SOS complex around 220 nm is considerably smaller than that of ordinary α-helical PLL.[1,2,8,9] It is not clear whether this is due to an environment effect of the bound surfactant ions on the rotational strength of the n-π^* transition as pointed out by Grourke and Gibbs[10] and Satake and Yang,[3] or incomplete helix formation due to very low SOS concentration.

Figure 2 shows the dependence of residue ellipticities, $[\theta]_{192}$ and $[\theta]_{221}$ on SOS concentration. PLL is seen to be α-helical only in the range 4.0—6.0 mM SOS, taking disordered structure and β-structure below and above the SOS concentration range, respectively.

The conformational change of the PLL induced by

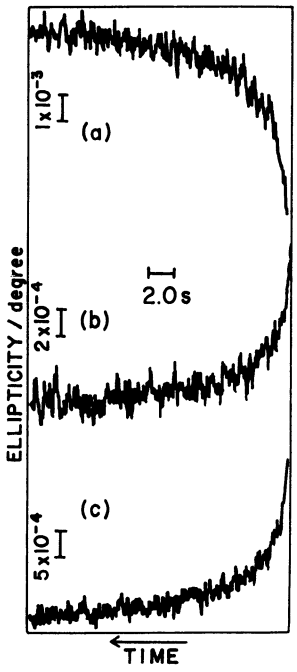

Fig. 3. Typical traces of ellipticity changes at 192 (a), 207 (b), and 221 nm (c) at 20 °C. The trace (a) is average of 32 repetitions and the traces (b) and (c) are averages of 8 repetitions. The final concentrations of PLL and SOS were 2.8×10^{-4} M and 6.0 mM, respectively.

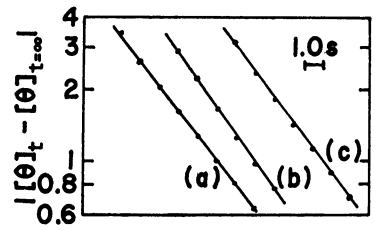

Fig. 4. Semilogarithmic first order plots for the approach to equilibrium on the three time courses in Fig. 3. The quantities representing $|[\theta]_t-[\theta]_{t=\infty}|$ are expressed in units in (a) 1×10^{-3}, (b) 2×10^{-3}, and (c) 5×10^{-4} degree, respectively, on the basis of best fit curve drawn on time course in each case.

SOS was followed by CD changes at 192 (wavelength at which the positive maximum appears in the difference CD spectrum directly obtained by use of a data processor; dotted curve III, Fig. 1), 207 and 221 nm (wavelengths at which the negative maximum appears in SOS solution, Fig. 1). Typical time courses are given in Fig. 3. As anticipated from the changes in the CD spectra (Fig. 1), the ellipticity at 192 nm changed from negative to positive, the negative ellipticities at 207 and 221 nm increasing with time. Although only half of the total ellipticity change with time near new equilibrium was observed at each wavelength due to the large dead volume of the stopped-flow system, the directions of the ellipticity changes with time at the three wavelengths were in line with the changes expected from the CD spectra (Fig. 1). This result and the identical rate constants calculated at three wavelengths led us to conclude that the observed process is the conformational change of PLL from the disordered structure to the α-helix, free from any possible artifacts which might be occasionally observed in stopped-flow measurements.[11] The forward rate constant for the conformational change from the coil to α-helix was determined by assuming that the conformational change of PLL is a first order reaction. The first order plot gives a linear relationship between $|[\theta]_t - [\theta]_{t=\infty}|$ and time, t (Fig. 4). The time courses at the three wavelengths approximately gave the same rate constant, k being almost independent of the SOS concentration between 4.0 and 6.0 mM (Fig. 5).

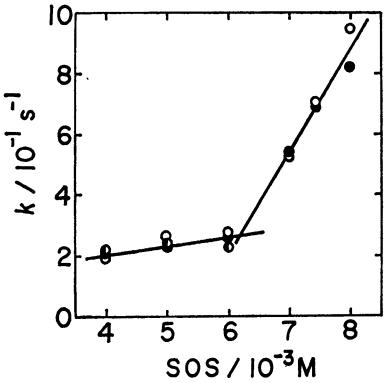

Fig. 5. Dependence of rate constant, k on SOS concentration at 20 °C. The rate constants were obtained from time courses at 192 (○), 207 (◐), and 221 nm (●). Final concentration of PLL was 2.8×10^{-4} M.

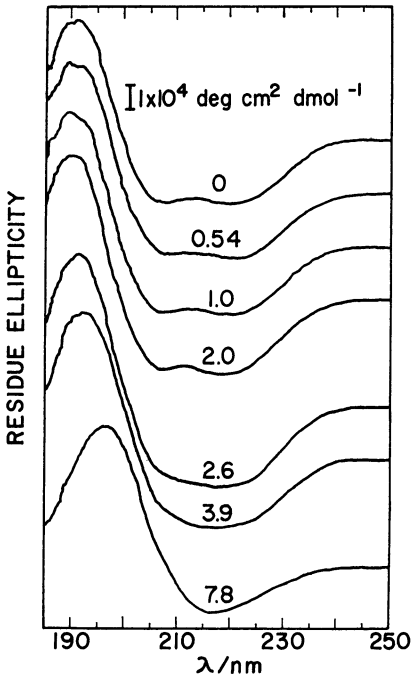

Fig. 6. Changes of CD spectrum of PLL in 5.0 mM SOS by addition of small amounts of 1-octanol at 20 °C. Numerical values in the figure denote concentrations of the 1-octanol in mM.

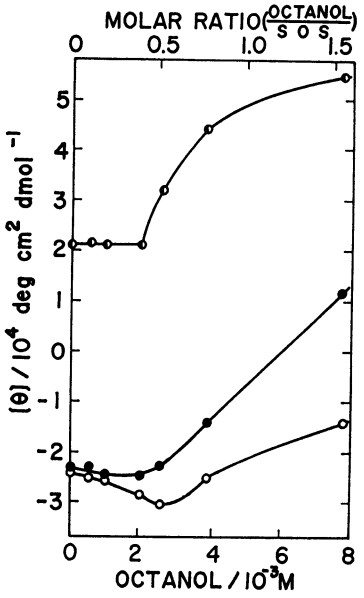

Fig. 7. Plots of $[\theta]_{197}$ (◐), $[\theta]_{207}$ (●), and $[\theta]_{221}$ (○) *vs.* concentration of 1-octanol and molar ratio of octanol/SOS at 20 °C. The concentration of SOS was 5.0 mM.

On the other hand, PLL adopts the β-structure above 7.0 mM SOS. Addition of a small amount of 1-octanol to α-helical PLL in 4.0—6.0 mM SOS caused the β-structure as shown in Fig. 6. The residue ellipticities at 197, 207, and 221 nm, $[\theta]_{197}$, $[\theta]_{207}$, and $[\theta]_{221}$ *vs.* 1-octanol and 1-octanol/SOS molar ratio plot is shown in Fig. 7. Above the octanol/SOS molar ratio=0.5, $[\theta]_{197}$, $[\theta]_{207}$, and $[\theta]_{221}$ gradually change with increase in the ratio of octanol/SOS. The PLL conformation is in a delicate balance between the coil, α-helix, and β-structure state. A slight change in circumstances may cause the interchange among the three states. Bound SOS neutralizes the charges on PLL, giving rise to ordered structures, especially α-helix at first. Increased degree of binding gives more hydrophobic atmosphere to the host polymer, the β-structure being enhanced. Addition of octanol also gives the α-helical PLL–SOS complex a more hydrophobic atmosphere, making it adopt the β-structure. The tendency that more hydrophobic environment favors β-structure is seen in Satake and Yang's study in which alkyl sulfates longer than SOS exclusively bring about the β-structure.[3] Alkyl sulfates bound to the charged sites on polymer would be in contact with each other more efficiently on the β-structure than the α-helix, since the alkyl sulfates are implanted radially on the α-helix, while they are oriented in the same direction on the β-structure. Such a difference in mutual contact is also reflected on a binding isotherm which is much steeper for the β-structured polypeptide than the α-helical one.[12]

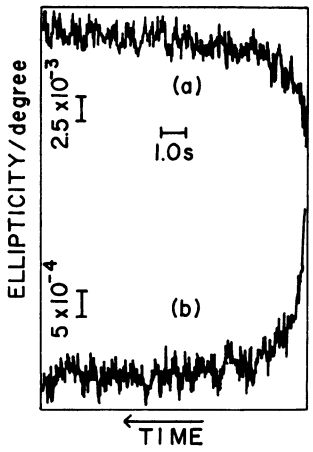

Fig. 8. Typical traces of ellipticity changes at 192 (a) and 221 nm (b) at 20 °C. The traces (a) and (b) are averages of 16 and 8 repetitions, respectively. The final concentrations of PLL and SOS were 2.8×10^{-4} M and 7.5 mM, respectively.

The ellipticity changes with time were observed above 7.0 mM SOS (Fig. 8). The change seems to be due to the conformational change of PLL from coil to β-structure, the rate constant sharply increasing with SOS concentration (Fig. 5).[13] There is no possibility that PLL passes through the α-helical state before attaining β-structure in the higher SOS concentration range, since no corresponding process was observed in such time courses as shown in Fig. 8. The disordered structure of PLL directly turned to the β-structure.

The octanol-induced conformational change of PLL was followed by the CD stopped-flow method. Typical time courses are shown in Fig. 9. The directions of the ellipticity changes in this case, as expected from a comparison of curve II with curve IV in Fig. 1, were reversed in contrast with those in the conformational change from the coil to α-helix or β-structure. Most of the total ellipticity change could be observed through-

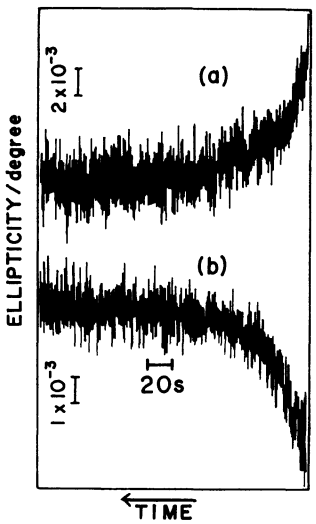

Fig. 9. Typical traces of ellipticity changes at 192 (a) and 221 nm (b) at 20 °C. This is the case of mixing PLL solution containing 5.0 mM SOS with a solution of 5.0 mM SOS containing slight amounts of 1-octanol. The final concentrations of the PLL and the octanol were 2.8×10^{-4} M and 7.8 mM, respectively. The traces (a) and (b) are averages of 8 and 2 repetitions, respectively.

out the time courses at these wavelengths, since the rate was one order of magnitude lower than those of the other two processes, the coil to α-helix or β-structure. The observed processes in Fig. 9 reflect the conformational change of PLL, from the α-helix to β-structure. The time courses at 192 and 221 nm gave an identical rate constant, $7.2\times10^{-2}\,s^{-1}$ at 20 °C. Such a slow process would be responsible for destruction-reconstruction mechanism: α-helix is unfolded and then the highly ordered β-structure is constructed. The conformational change of PLL from coil to β-structure was also observed in mixing the PLL solution with 5.0 mM SOS solution containing 7.8 mM 1-octanol (both final concentrations). The observed time courses in this case also gave a similar rate constant, $5.5\times10^{-1}\,s^{-1}$, to those obtained in the conformational change from coil to β-structure in the presence of 7.0 mM SOS. The process may proceed without passing through α-helix, implying lack of destruction of α-helix.

The rate constants for three kinds of conformational changes of PLL in SOS solution seem to be low as compared with the reported values for conformational changes of polypeptides.[14] The difference is probably due to the characteristic situation in surfactant solutions as follows. The binding of SOS to PLL causes a discharge of the cationic groups of PLL, but the amount of bound SOS is not enough to discharge all of them. The surfactants are forced to move from the first binding sites to other vacant sites with the progress of conformational change of the polypeptide, since the bulky hydrophobic groups of surfactants should find minimal free energy in order to reconcile the structure formation with maximal contact between the hydrophobic groups of bound surfactants and the hydrophobic parts of the polypeptide. Such rearrangements of bound surfactants to the polypeptide require a much longer time in comparison with conformational change induced by proton which is more effective than the surfactant in reactivity and mobility because of its small size. The conformational change of polypeptide in the surfactant solution seems to occur accompanied by strong interaction.

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