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Conformation Analyses of Poly(L-lysine) Induced by Various Surfactants

Kunio Takeda
Department of Applied Chemistry, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700

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The relative proportion of α-helix, β, and random-coil structures in each conformation of poly(L-lysine) (PLL) caused by surfactants was computed by simulating a mixed spectrum of typical circular dichroism(CD) spectra for these structures to the experimentally obtained spectrum. Simulation was done in both the 190—250 nm and 200—250 nm regions at 1-nm intervals. The results show that PLL assumed 52 and 75% α-helix (at largest) in sodium octyl sulfate (SOS) and sodium 1-octanesulfonate solutions, respectively. The α-helical structure was found to coexist with considerable amounts of β and random-coil structures in these surfactant solutions. For instance, 51% α-helical structure coexisted with 29% β-structure and 20% random-coil structure in 6.0 mmol dm^{-3} SOS. In contrast, PLL did not assume any α-helical structure in the concentration range of 0 to 10 mmol dm^{-3} sodium dodecyl sulfate(SDS). Rather, the random-coil structure was transformed to a β-structure when SDS was added to PLL. The effects of NaCl and of 1-octanol on the relative proportions of the three structures in PLL were also examined.

Conformational changes of homopolypeptides have been extensively studied using circular dichroism (CD) measurements. Poly(L-lysine) (PLL) has been frequently selected as a typical polypeptide because of its diversity in conformations.[1–8] The conformational change of PLL induced by surfactants has been studied by a number of investigators.[3–8] The conformation of PLL transforms to α-helix and then β-structure with an increase of sodium octyl sulfate (SOS)[3,7], while it transforms to the β-structure and then to the α-helical structure with an increase of sodium 1-octanesulfonate.[6] The PLL assumes a β-structure in solutions of sodium alkyl sulfates with hydrophobic chains longer than ten carbons.[3–5,8] However, few attempts have been made quantitatively to estimate the relative proportions of α-helix, β, and random-coil structures in the conformations of homopolypeptides induced by surfactants.

The CD spectra of the various structural conformations in a polypeptide chain are assumed to be additive.[9–18] This is now commonly accepted even for proteins which have more complex conformations than the homopolypeptides.[9–18] The relative proportion of α-helix, β, and random-coil structures can be estimated from the experimentally obtained spectrum of a polypeptide with known reference spectra of the three structures. In the present study, the relative proportion of the three structures in PLL has been computed at various concentrations of the three surfactants, SOS, sodium 1-octanesulfonate, and sodium dodecyl sulfate (SDS). This has been achieved by simulating a mixed spectrum of the typical CD spectra for these structures to the experimentally obtained spectrum according to the method of Greenfield and Fasman.[9]

Experimental

The source of PLL has been described elsewhere.[7] The SOS and SDS used were the same samples as used before.[8,19] The 1-octanol was purchased from Aldrich Chemical Co. (purity: 99%).

CD measurements were made with a JASCO J-500A spectropolarimeter equipped with a DP-501 data processor. Details of the CD measurements have been described previously.[7] All measurements were carried out at 20 °C and at a neutral pH.

Results and Discussion

The CD spectrum is a curve of continuous values of the residue ellipticity of structural elements averaged over a certain wavelength region. By examining the shape and strength of the CD spectrum over the region of π-π* and n-π* transitions,[9] the relative proportions of α-helix, β, and random-coil structures could be obtained on each conformation of PLL which was considered to partially assume these three structures in surfactant solutions.[3–8]

It has been found that PLL assumes an α-helical structure only in solutions of SOS[3,7] and sodium 1-

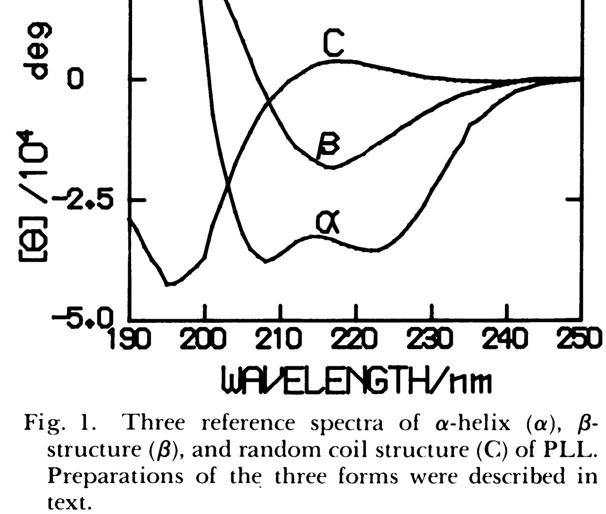

Fig. 1. Three reference spectra of α-helix (α), β-structure (β), and random coil structure (C) of PLL. Preparations of the three forms were described in text.

octanesulfonate⁽⁶⁾ which have the same hydrophobic chain of 8 carbons. The CD spectra of PLL, in SOS concentrations between 4 and 6 mM (1 M=1 mol dm^{-3}), show a double-negative maximum which is indicative of the α-helix.⁽⁷⁾ PLL also displays two types of CD spectra which characterize random-coil and β structures below and above the concentration range of SOS, respectively.⁽⁷⁾ In sodium 1-octanesulfonate solutions, the CD spectra of PLL are dependent on the concentration. At concentrations of the surfactant below 6 mM, they are characteristic of a random conformation, of the β-structure between 6 and 8 mM, and of the α-helix structure above 8 mM.⁽⁶⁾

Figure 1 shows three reference CD spectra⁽⁹⁾ corresponding to the three structures. The reference spectra were used to analyse the experimentally obtained spectrum of PLL. The spectrum of the α-helical structure was determined at a concentration of 0.56 mM PLL (residue) at pH 11.6. The same sample of PLL was heated to 52 °C for 15 min and then cooled to obtain the spectrum of the β-structure.⁽²⁰⁾ The spectrum of PLL obtained at a neutral pH in the absence of a surfactant was used as a typical spectrum of the random-coil structure. The negative intensities of the π-π^* and n-π^* transitions of the α-helical PLL in surfactant solutions (Fig. 1 in Ref. 3 and Fig. 1 in Ref. 7) are considerably smaller than that of the polypeptide in the alkaline pH range (Fig. 1). This was first observed by Grourke and Gibbs with a CD spectrum of poly(L-ornithine) in the α-helical state in SDS.⁽²¹⁾ It is not clear whether this is due to an environmental effect of the surfactant ions on the rotational strength of the two transitions or due to an incomplete α-helix formation. However, when PLL adopts a β-structure in SDS, the positive intensity of the π-π^* transition becomes larger⁽⁸⁾ than that of a typical reference spectrum (Fig. 1). The intensities of both transitions are somewhat less in β-structured PLL in SOS⁽⁷⁾ than in SDS⁽³,⁸⁾ in spite of the fact that SOS has a shorter chain length than SDS. The environmental effect is expected to decrease with a decrease in the chain length of the surfactant. Furthermore, the CD spectra of α-helical PLL shows stronger negative intensities in sodium 1-octanesulfonate⁽⁶⁾ than in SOS,⁽⁷⁾ although both have the same chain length. These facts are not sufficient to conclude that there is no appreciable environmental effect on the two transitions, but lead us to suspect that the low intensities of the CD spectra are due to an incomplete formation of the ordered structures in these surfactant solutions. Then, it is assumed that surfactant ions have little effect on the rotational strengths of the transitions. This assumption is also supported by the results of the present computation on the relative proportions of the three structures. This will be discussed further.

If PLL takes either of the three structures—α-helix, β, and random-coil structures, the observed residue ellipticity, $[\theta]_{\mathrm{obsd}}$, is represented by

$$[\theta]_{\mathrm{obsd}} = f_\alpha[\theta]_\alpha + f_\beta[\theta]_\beta + f_c[\theta]_c$$

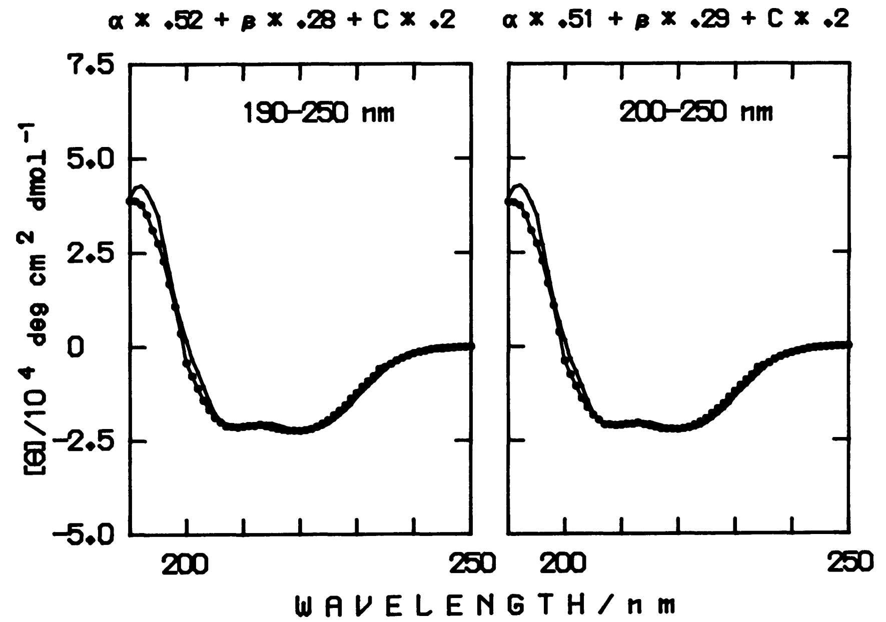

Fig. 2. The computed (—●—) and the experimental (——) CD spectra of PLL in 6.0 mM SOS. The concentration of PLL was 0.56 mM (residue). The simulation was done in two wavelength regions of 190—250 nm (left) and 200—250 nm (right). In the simulation done in 200—250 nm (right), the spectrum was calculated also in the range of 190—200 nm using the relative proportions of the three structures determined in 200—250 nm. The formula on each figure indicate that the computed spectrum consists of the three structural elements with given fractions.

where $[\theta]_\alpha$, $[\theta]_\beta$, and $[\theta]_c$ are the residue ellipticities of the reference spectra of the α-helix, β, and random-coil structures, respectively and f_α, f_β, and f_c are the relative proportions of the corresponding structures. No constraints are imposed on the relative proportions, f_i, except that they add up to unity: $\sum f_i = 1$. The computation was done in the 190—250 nm and 200—250 nm regions at 1-nm intervals by varying the amounts of each structure at 1% intervals. Figure 2 shows simulations of CD spectra of PLL which had a double-negative maximum indicative of the α-helical structure. The results indicate that the PLL contains 51—52% α-helix, 28—29% β-structure, and 20% random-coil structure. Figures 3 and 4 present the simulated spectra when PLL adopted predominantly β and random-coil structures, respectively. There is a considerable difference between the relative proportions obtained by the simulations in the 190—250 nm and 200—250 nm regions in Fig. 4. In this case, the greatest difference appeared between the two simulated spectra over the wavelength regions of this investigation. This might be due to the assumption used here that only the three structures are adopted. It appears possible that PLL may also contain slight amounts of structures other than these three since the cationic PLL is not fully neutralized in low concentrations of the surfactant. The simulation was conducted in the two wavelength regions stated above. It is well known that the signal-to-noise ratio decreases with a decrease in wavelength, especially below 200 nm. Therefore, if possible, the simulation should be done above 200 nm. However, when PLL predominantly assumes a random-coil structure, it shows a distinct spectrum only in the region below 210 nm. The values computed in the 190—250 nm region were useful in the case of PLL rich in the random-coil structure. When PLL assumes α-helix and β-structure (both of which show characteristic CD curves in the 200—250 nm region), the relative proportions computed in this wavelength region are considered to be reliable.

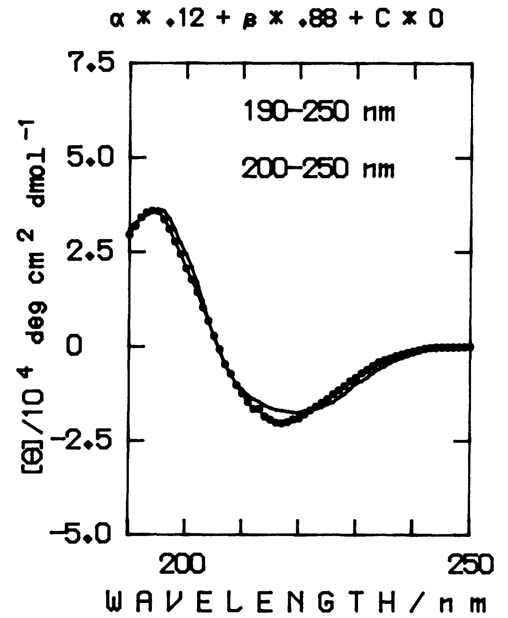

Fig. 3. The computed (—●—) and the experimental (——) CD spectra of PLL in 8.0 mM SOS. The simulations in both wavelength regions of 190—250 nm and 200—250 nm gave the same relative proportions in SOS of this concentration.

Figure 5 shows changes in the relative proportions of the three structures as a function of SOS concentration. As can be seen, PLL assumes an α-helical structure between 2 and 9 mM SOS. The α-helix content was around 52% in 4 to 6 mM SOS. The content of the β-structure increased with an increase in SOS concentration, while that of the random-coil structure decreased with an increase in surfactant concentra-

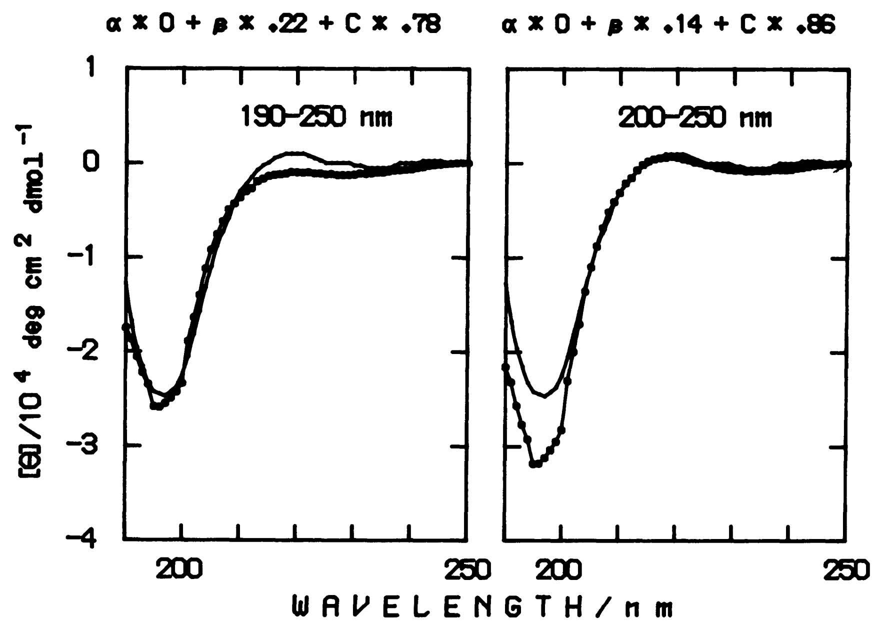

Fig. 4. The computed (—●—) and the experimental (——) CD spectra of PLL in 2.0 mM SOS.

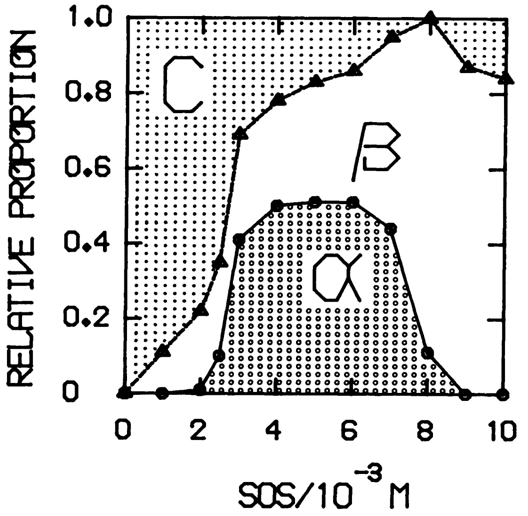

Fig. 5. The relative proportions of α-helix (α), β-structure (β), and random coil structure (C) of PLL as a function of SOS concentration. The boundary between the α-helix and the β-structure is plotted (—○—) and that between the β-structure and the random coil done (—△—). Then each area gives the fraction of the corresponding structure. The same method was used in following figures.

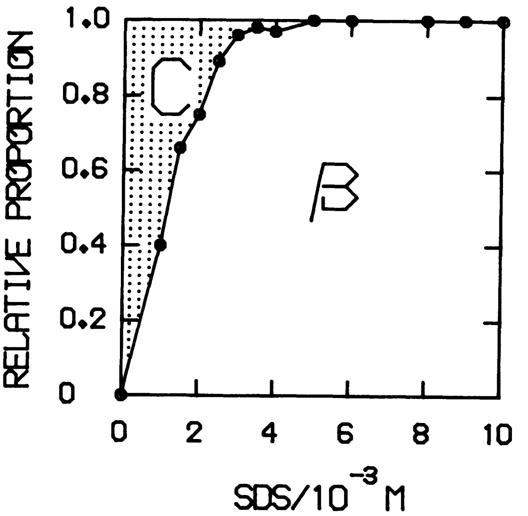

Fig. 6. The relative proportions of β-structure (β) and random coil structure (C) of PLL as a function of SDS concentration. The simulation was done by the same program as applied to the case of PLL in SOS solutions. The α-helix fraction was zero over the entire SDS concentrations in both the computations in 190—250 and 200—250 nm.

tion. The relative proportion of the β-structure and the random-coil structure was 29—35% and 26—14%, respectively in an SOS concentration range of 4 to 6 mM, while PLL assumed predominantly the α-helix structure in this range. Simulation was done for the CD spectra measured in 0.14 to 0.79 mM PLL (residue). However, a change in the concentration of PLL did not have any effect on the relative proportions of the three structures.

PLL assumes a β-structure in solutions of sodium decyl sulfate[5] and SDS.[3,4,8] Figure 6 shows changes in the relative proportions of the α-helix, β, and random-coil structures in an SDS solution. The computation was carried out by a similar simulation method as was done for the PLL conformation in SOS to obtain the relative proportions of the three structures. The α-helix content was negligible over the entire range of SDS concentration. The β-structure content increased with an increase in SDS concentration upto 5.0 mM and finally attained 100%. If there is an environmental effect of surfactant ions to decrease the CD intensities,[21] we cannot estimate 100% proportion of the ordered structure in surfactant solutions without taking into account the fading of the CD spectra. Nevertheless, the present simulation shows that PLL assumes a 100% β-structure in SDS solutions. This result also indicates that there is no appreciable environmental effect of surfactant ions on the CD intensities.

Simulation was also done on the CD spectra of PLL in sodium 1-octanesulfonate solutions containing 21.3 mM NaCl measured by Hayakawa *et al.*[6] Table 1 presents the relative proportions of the three structures in conformations of PLL caused by sodium 1-octanesulfonate. The result shows that the random-coil structure of PLL is sharply transformed to the β-structure between 5.57 and 6.07 mM of the surfactant and the β-structure content decreased above 8.30 mM. The α-helical structure is formed above 8.10 mM of the surfactant. At a surfactant concentration of 10.1 mM where the residue ellipticity at 222 nm becomes constant (see Fig. 2 in Ref. 6), 75% α-helix coexists with 11% β-structure and 14% random-coil. It is interesting to note that the coil to β-structure transition occurs predominantly in lower concentrations of sodium 1-octanesulfonate and the coil to α-helix transition in higher concentrations, in contrast to the transitions in

Table 1. Relative proportion of α-helix, β-structure, and random-coil structure of PLL in sodium 1-octanesulfonate solutions[a)]

Concentration of sodium 1-octanesulfonate/mM	α-Helix	β-Structure	Random-coil	Simulated region of wavelength/nm[b)]
5.57	0	0.04	0.96	205—250
6.07	0	0.70	0.30	205—250
7.79	0	0.71	0.29	207—250
8.10	0.20	0.68	0.12	205—250
8.30	0.52	0.40	0.08	204—250
10.1	0.75	0.11	0.14	198—250

a) Computed for CD spectra measured by Hayakawa *et al.*[6] b) Restricted by wavelength region presented in Ref. 6.

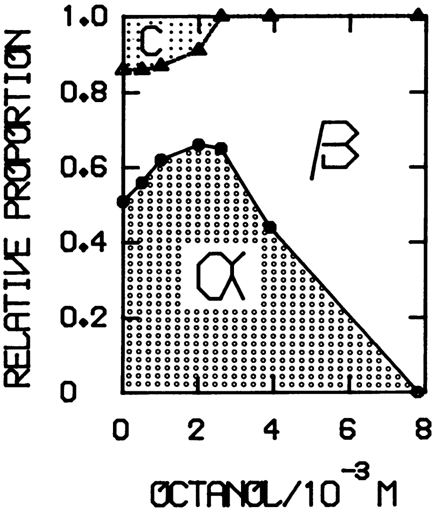

Fig. 7. The relative proportions of α-helix (α), β-structure (β), and random coil structure (C) of PLL as a function of 1-octanol concentration in the presence of 6.0 mM SOS. The concentration of PLL was 0.56 mM (residue).

TABLE 2. CHANGE IN RELATIVE PROPORTION OF α-HELIX, β-STRUCTURE, AND RANDOM-COIL STRUCTURE OF PLL BY ADDITION OF NaCl[a)]

Concentration of NaCl/mM	α-Helix	β-Structure	Random-coil
0	0.51	0.26	0.23
5.0	0.50	0.27	0.23
10.0	0.35	0.42	0.23
15.0	0.32	0.47	0.21
20.0	0.25	0.58	0.17
25.0	0.26	0.59	0.15
30.0	0.02	0.68	0.30

a) Values simulated in 200—250 nm region.

SOS. It has been reported that PLL assumes an α-helical structure only in SOS[7)] and sodium 1-octanesulfonate[6)] solutions of a neutral pH. However, it is not clear to what degree the α-helical structure is formed. An important result of the present simulation is the knowledge that the α-helical structure coexists with the other two structures in both the surfactant solutions.

The effect of the addition of 1-octanol on the relative proportions of the three structures was examined on the conformation of PLL in 6.0 mM SOS. Figure 7 shows the changes in relative proportions of the three structures as a function of 1-octanol concentration. The α-helix content increased until 2.0 mM octanol and abruptly decreased beyond 2.6 mM. A small amount of octanol seems to provide the best condition to form the α-helical structure, but a greater hydrophobic atmosphere enhances the β-structure. The content of the random-coil structure decreased from 14% to 0% upto 2.6 mM octanol. Therefore, only the α-helix and β-structure exist above 2.6 mM octanol in the presence of 6.0 mM SOS.

We have previously found that the double-negative maximum of PLL, which is a characteristic of the α-helical structure, fades out with an increase in the added NaCl concentration in an SOS solution (Fig. 4 in Ref. 8). These fading CD spectra of PLL by the addition of NaCl were simulated in the same manner. The results are shown in Table 2. The presence of 20—25 mM NaCl reduces the α-helix content to approximately half of that found in the absence of the electrolyte. The content of the random-coil structure was essentially constant below 25 mM of NaCl. Therefore, it appears that the addition of NaCl changes the relative proportions of only the α-helix and β-structure. In the presence of about 20 mM NaCl, the α-helix content of PLL in SOS is one third of that in sodium 1-octanesulfonate. The present result indicates that in addition to the chain length of the hydrophobic group and charge of the hydrophilic group of surfactant, the structural features of the hydrophilic group are also important for the induction of the α-helical structure.

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