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Helix Formation of Poly(L-ornithine) in Decyl and Dodecyl Sulfate Solutions

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Synopsis. Structural changes in poly(L-ornithine)[(Orn)$_n$] were studied in solutions of sodium decyl sulfate (NaDecSO$_4$) by the curve-fitting method of circular dichroism. In the NaDecSO$_4$ concentration range from 4 to 9 mmol dm^{-3}, only the β-structure was found. Below and above this concentration range, however, the α-helical structure was formed slightly, its fraction being less than 20%. The addition of 1-octanol enhanced the helix formation in the (Orn)$_n$-NaDecSO$_4$ complex.

Many investigations have been conducted in the past three decades that have dealt with the conformational changes in homopolypeptides such as poly(L-ornithine)[(Orn)$_n$] homologs in anionic surfactant solutions.[1—13] However, few attempts have been made to estimate quantitatively the presence of α-helix, β-structure, and random coil in these homologs in the surfactant solutions. Recently, we computed the fractions of α-helix, β-structure, and random coil of (Orn)$_n$ in a sodium dodecyl sulfate (NaDodSO$_4$) solution[12] using a simulation of the circular dichroism (CD) spectra similar to Greenfield and Fasman.[14] This method may make possible a quantitative study when a slight amount of helical structure in the polypeptide chain is induced by another surfactant solution. We now report proportional changes of α-helix, β-structure, and random coil in (Orn)$_n$ induced by sodium decyl sulfate (NaDecSO$_4$), together with the effect of added 1-octanol on the formation of helical structure in the same surfactant solution.

Experimental

The (Orn)$_n$ sample employed was described in a previous study.[12] The NaDecSO$_4$ was prepared from 1-decanol (purity: 99.8%), essentially according to the method of Dreger et al.[15] The critical micelle concentration of the surfactant, as determined by the electric conductivity method, was 31.5 mM (1 M=1 mol dm^{-3}) at 25 °C.

The CD measurements were carried out with a Jasco J-500A spectropolarimeter equipped with a Jasco DP-501 data processor. Details of the experimental procedures have been described elsewhere.[10,12] The CD measurements were made under the same conditions as have been described previously: temperature, 20 °C; pH, neutral; the residue concentration of (Orn)$_n$, 9.2×10^{-5} M.[12] The simulation of the CD spectrum was made over the wavelength region of 190—250 nm with the assumption that the (Orn)$_n$ assumes only three structures: α-helix, β-structure, and random coil. The relative proportions of these three structures were calculated by the same method as in a previous study.[12]

Results and Discussion

The CD spectra of (Orn)$_n$ at various NaDecSO$_4$ concentrations are shown in Fig. 1. Figure 1 also shows two CD spectra of the polypeptide in a 0.8 mM NaDecSO$_4$ solution containing slight amounts of 1-octanol. The addition of 1-octanol enhanced helix formation in (Orn)$_n$, as can be seen by the increase in the magnitude of the α-helical-type CD spectrum. In the NaDecSO$_4$ concentration range from 4 to 9 mM, the surfactant solution containing (Orn)$_n$ became slightly turbid, but this turbidity did not interfere in the CD measurements (the same phenomenon is observed in the NaDodSO$_4$.[12] In this concentration range, the CD spectra of (Orn)$_n$ were typically of β-structure (not shown, refer to Fig. 1 in Ref. 12). These CD spectra were analyzed using the same reference spectra as in the previous study.[12] The proportional

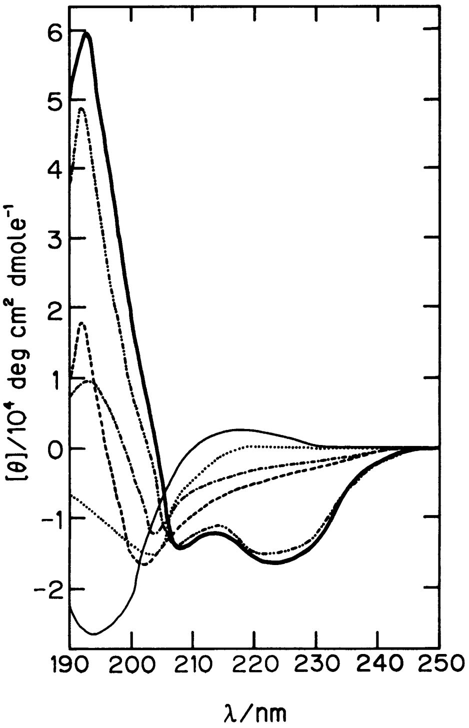

Fig. 1. CD spectra of (Orn)$_n$ in NaDecSO$_4$ solutions in the absence and the presence of 1-octanol. NaDecSO$_4$ concentrations in the absence of octanol: 0.3 (—); 0.4 (······); 0.8 (–·–·–) and 14 mM (-----). Added octanol concentrations to 0.8 mM NaDecSO$_4$ solution: 1.02 (–··–··–) and 1.28 mM (——). The polypeptide concentration: 9.2×10^{-5} M (residue). The CD spectra of (Orn)$_n$ were time-dependent in the order of hour in 0.4 and 0.8 mM NaDecSO$_4$ solutions without the octanol. All the measurements of spectra were carried out within 10 min after mixing the (Orn)$_n$ solution with the surfactant solution.

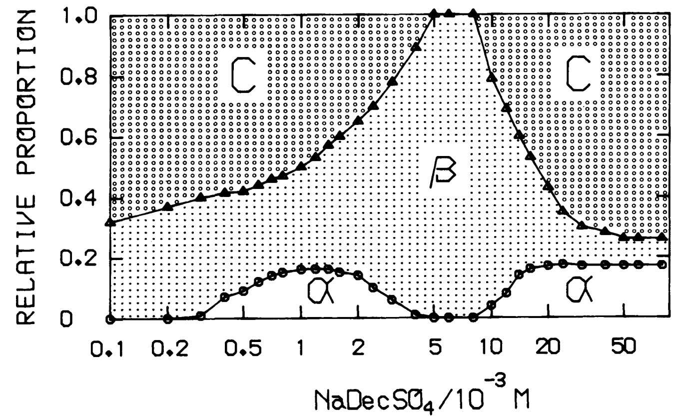

Fig. 2. Proportional changes of α-helix (α), β-structure (β), and random coil (C) in $(Orn)_n$ as a function of $NaDecSO_4$ concentration. The proportions were estimated using the CD spectra measured under the same conditions as mentioned in Fig. 1. The boundary between the α-helix and the β-structure 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 the following figure.

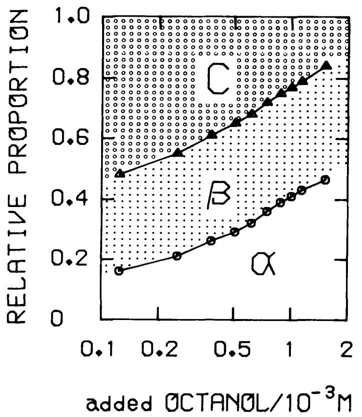

Fig. 3. Proportional changes of α-helix, (α), β-structure (β), and random coil (C) in $(Orn)_n$ as a function of added 1-octanol to 0.8 mM $NaDecSO_4$ solution.

changes in the secondary structure of $(Orn)_n$ as a function of concentration of $NaDecSO_4$ are presented in Fig. 2. As is seen, $(Orn)_n$ assumes a 100% β-structure in the $NaDecSO_4$ solutions of 5 to 9 mM. The data in this figure also show that the helical structure is assumed in two concentration ranges of $NaDecSO_4$; between 0.3 and 4 mM and above 9 mM $NaDecSO_4$. The $(Orn)_n$ assumes the helical structure also in two concentration ranges of $NaDodSO_4$.[12] However, higher concentrations of $NaDecSO_4$ were required than was the case with $NaDodSO_4$.[12] In the lower concentration range of $NaDodSO_4$, the polypeptide assumes an unstable helix. This unstable helix changes to β-structure within a matter of minutes.[12] The helical structure formed in the lower concentrations of $NaDecSO_4$ was also unstable, but not to the same extent; several hours were required for the change to the β-structure. The helical proportion was much lower in $NaDecSO_4$ than in $NaDodSO_4$; the proportion was less than 12 and 17% in the two $NaDecSO_4$ concentration ranges as seen in Fig. 2, whereas the maximal proportions of the unstable and the stable helical structures in $NaDodSO_4$ are 50 and 73% respectively.[12]

This difference between the helical proportions of $(Orn)_n$ formed in the two surfactant solutions must be necessarily due to the difference in the number of carbons in the hydrophobic portion of the two surfactants. As is seen in Fig. 1, the presence of slight amounts of octanol in 0.8 mM $NaDecSO_4$ greatly enhanced the double-minimum of the helical-type CD spectrum of $(Orn)_n$. The proportional changes in the three structures are plotted as a function of 1-octanol concentration in Fig. 3. The helical proportion increased to 46%, which is comparable with the maximal fraction of the unstabale helix in $NaDodSO_4$.[12] The increment of hydrophobicity due to 1-octanol (which probably leads to the formation of mixed micelle of $NaDecSO_4$ with 1-octanol) clearly correlates with the increase in helical proportion. The proportion of β-structure, however, was not affected by the addition of 1-octanol (Fig. 3). A similar increase in the helical proportion of $(Orn)_n$ was observed by the addition of 1-octanol to $NaDecSO_4$ solutions at other concentrations. For example, the addition of 2 mM 1-octanol increased helical proportion from 4 to 12% in 0.4 mM $NaDecSO_4$.

The positive charges in the side groups of $(Orn)_n$ or poly(L-lysine)[$(Lys)_n$] are generally considered to be eliminated by the electrostatic interaction with the hydrophilic groups of anionic surfactant ions such as alkyl sulfate ions.[4,7] The neutralization of the charges of polypeptide by the surfactant ions, however, is not enough to cause the formation of α-helix and β-structure, as is expected from the previous studies of the effect of the hydrocarbon chain length of a surfactant.[4,10,11] It is reasonable to speculate that the hydrocarbon chain may be involved in the formation of secondary structure in the surfactant–polypeptide complexes. Tseng and Yang have suggested that the hydrocarbon chains of surfactants bound to polypeptide might be juxtapositioned to other hydrocarbon chains of the excess surfactant molecules, thus forming a micelle-like structure.[7] In this regard, it is worth noting that the proportion of β-structure of $(Orn)_n$ reaches 100% in $NaDecSO_4$ as well as in $NaDodSO_4$.[12] $(Lys)_n$ also assumes 100% β-structure in $NaDodSO_4$.[13] In the antiparallel β-sheet structure, the polypeptide chains run in opposite directions, being hold together by hydrogen bonds of peptide linkage with the amino acid side groups alternatively sticking up and sticking down (see Fig. 6 in Ref. 16). This means that the alkyl sulfate ions bound to the side groups of β-structural polypeptide stretch out perpendicularly to both the polypeptide backbone and the hydrogen bonds. When such a β-sheet is formed, the alkyl chains of surfactants bound to the residues might stand close together on both sides of the sheet.

This might lead to the formation of the McBain lamellar micelle[17) with alkyl chains. These alkyl chains may belong to the excess surfactant molecules or to the surfactants bound to other β-structural polypeptides (or to other distant β-structural segments of the identical polypeptide). As mentioned above, when $(\mathrm{Orn})_n$ and $(\mathrm{Lys})_n$ predominantly assume the β-structure in these surfactant solutions, the solutions become slightly turbid. The turbidity might be due to the accumulation of the sheets through the formation of the lamellar micelle. On the other hand, when the polypeptide assumes the α-helical structure, the side groups of amino acids are considered to stretch out in every direction around the helical rod.[18) This feature suggests that alkyl sulfate ions bound to side groups of α-helical polypeptide might stretch out in every direction in a different manner from those bound to β-structural polypeptides. How these complexes assume the α-helical structure remains unknown.

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