EFFECT OF CHAIN LENGTH AND CONCENTRATION OF ANIONIC SURFACTANTS ON THE CONFORMATIONAL TRANSITIONS OF POLY(L-ORNITHINE) AND POLY(L-LYSINE) IN AQUEOUS SOLUTION[1]

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SUMMARY: The conformation of poly(L-ornithine)(PLO) and poly(L-lysine)(PLL) in solutions of sodium alkyl sulfates, $CH_3(CH_2)_nSO_4Na$ with n = 7, 9, 11, 13 and 15 was studied by circular dichroism.[3] PLO adopts a helical conformation in all 5 homologs and PLL a β-form in only 4 of the homologs. With octyl sulfate PLL has a helical conformation instead. These conformations were observed in solution of surfactants both below and above the critical micelle concentration.

Surfactants have been extensively used in biochemical preparations and they are classified as effective denaturants of proteins at remarkably low reagent concentrations. However, the mechanism of their action on protein conformation remains unclear. It has been inferred from optical rotation studies that denaturation by SDS[3] of ovalbumin (1) and β-casein, histone fraction F_1 and chymotrypsinogen (2) may actually result in forming new helical regions that are not present in the native molecule. The CD study of model polypeptides in SDS solution led to the interesting finding that PLO undergoes a coil-to-helix transition (3) and PLL a coil-to-β transition (4), noting that PLO has one methylene group less in the side chain than PLL. In this communication we report that the polypeptide conformation in surfactant

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[3]Abbreviations used in this work: SDS, sodium dodecyl sulfate; SOS, sodium octyl sulfate; SDeS, sodium decyl sulfate; STS, sodium tetradecyl sulfate; SHS, sodium hexadecyl sulfate; CMC, critical micelle concentration; PLO, poly(L-ornithine); PLL, poly(L-lysine); CD, circular dichroism.

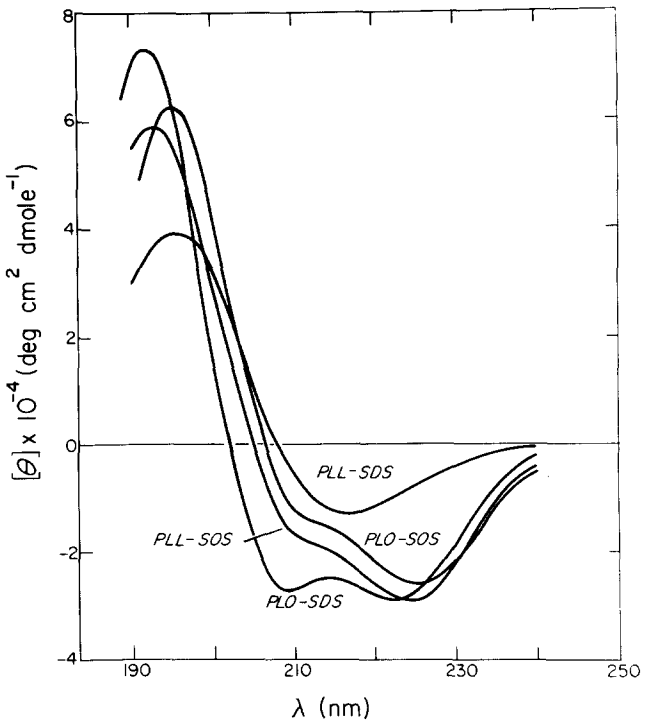

Figure 1. The CD of poly(L-ornithine)(PLO) and poly(L-lysine)(PLL) in sodium dodecyl and octyl sulfates (SDS and SOS) at 25°C. PLO-SDS, 1.1 x 10^{-4} M/0.026 M; PLO-SOS, 5.4 x 10^{-5} M/0.68 M; PLL-SDS, 1.2 x 10^{-4} M/0.026 M; PLL-SOS, 6.2 x 10^{-5} M/0.70 M.

solution can be influenced by the chain length of the surfactant. These conformational changes can occur in solution of surfactants below as well as above the CMC.

Effect of chain length of surfactants. Figure 1 shows the conformations of PLO and PLL in the presence of sodium octyl and dodecyl sulfates in neutral solution. The CD spectrum of the PLO-SDS complex is characteristic of a helical conformation with a double minimum at 209 and 222 nm and a maximum at 191-2 nm (our $[\theta]_{222}$ = -28600 deg cm^2 dmole^{-1} is about three times that reported by Grourke and Gibbs (3)). For PLO in SOS solution the 209-nm nimimum changes into a shoulder and the positions of the other two extrema are red-shifted by 3 nm. But the CD spectrum can still be regarded as the helical type. In striking contrast the conformation of PLL is different in the two surfactant solutions. The PLL-SDS complex has a CD spectrum

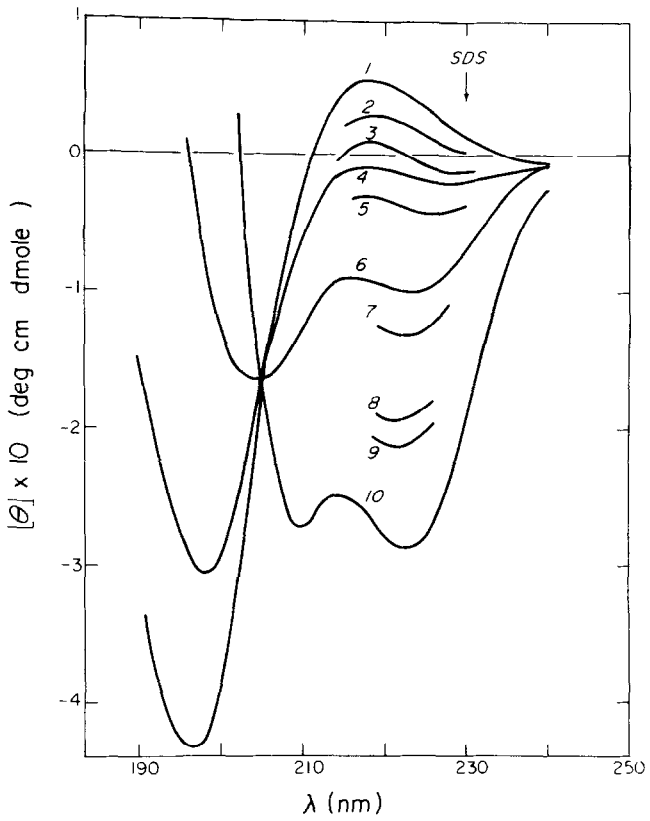

Figure 2. The CD of poly(L-ornithine) in sodium dodecyl sulfate solutions at 25°C. PLO: 1.08×10^{-4} M; SDS: 1, 0 M; 2, 2.45×10^{-5} M; 3, 3.40×10^{-5} M; 4, 4.90×10^{-5} M; 5, 5.96×10^{-5} M; 6, 1.02×10^{-4} M; 7, 1.19×10^{-4} M; 8, 1.53×10^{-4} M; 9, 1.70×10^{-4} M; 10, 2.55×10^{-2} M.

typical of a β-form with a minimum at 217-8 nm and a maximum at 195 nm (our $[\theta]_{217}$ = -13200 deg cm^2 dmole^{-1} can be compared with the literature of -9000 (4,5); we also found that the $[\theta]_{217}$ value dropped to -8000 after filtration through a Millipore filter of 5-μ pore size). But the CD spectrum of the PLL-SOS complex resembles that of the PLO-SOS complex in displaying a minimum at 225 nm with a shoulder near 210 nm and a maximum at 193 nm, suggesting the presence of a helical conformation. These findings should caution us against the use of impure surfactants in the study of protein conformation. For instance, some commercial SDS preparations contain in addition to dodecyl sulfate mixtures of lower and higher homologs.

Table I summarizes the numerical values of the CD extrema for PLO and

PLL in 5 surfactant solutions (above CMC). Their magnitude appears to decrease progressively with decreasing chain length of the surfactants, except when the polypeptide adopts a different conformation as in the case of the PLL-SOS complex. All 5 homologs of alkyl sulfates induce a coil-to-helix transition for PLO in aqueous solution, but the polypeptide appears to be less helical when the lower homologs are used. The $[\theta]_{222}$ of the PLO-SDS complex is considerably smaller (in magnitude) than that of helical PLL (6,7) or poly(L-glutamic acid)(7) in water (ca. -36000 to -40000 deg cm^2 $dmole^{-1}$). Grourke and Gibbs (3) have attributed this to an environment effect of the bound surfactant ions on the rotational strength of the n-π^* transition. Thus, it is difficult to make any quantitative estimate of the percentages of helix and unordered form in the complex. With the exception of octyl sulfate, the 4 alkyl sulfates promote a coil-to-β transition for PLL in neutral solution. Here again the percentage of β-form seems to decrease with decreasing chain length. The $[\theta]_{217}$ of the PLL-SDS complex is also smaller than that of β-PLL in water (6). We also observed that $[\theta]_{222}$ for PLO in SDS changed from -33700 at 9°C to -21200 deg cm^2 $dmole^{-1}$ at 45°C, suggesting a gradual breaking up of the helix at elevated temperature. On the other hand, $[\theta]_{217}$ for PLL in SDS remained virtually constant up to 35°C and rose (in magnitude) to -14300 deg cm^2 $dmole^{-1}$ at 45°C.

Effect of concentration of surfactants. Figures 2 and 3 show the changes in CD spectra of PLO and PLL with increasing surfactant concentrations, the polypeptide (residue) concentrations being kept constant. The coil-to-helix transition for PLO and coil-to-β transition for PLL seem to be almost linear with surfactant concentrations. Except for curves 10 in Fig. 2 and 8 in Fig. 3, the SDS concentrations used were well below the CMC. It is the ratio, R, of concentrations of surfactant to polypeptide (residue), not CMC, that determines the conformation of polypeptide-surfactant complexes. At R slightly larger than unity, the complexes were insoluble and their composition was stoichiometric (one surfactant ion per polypeptide

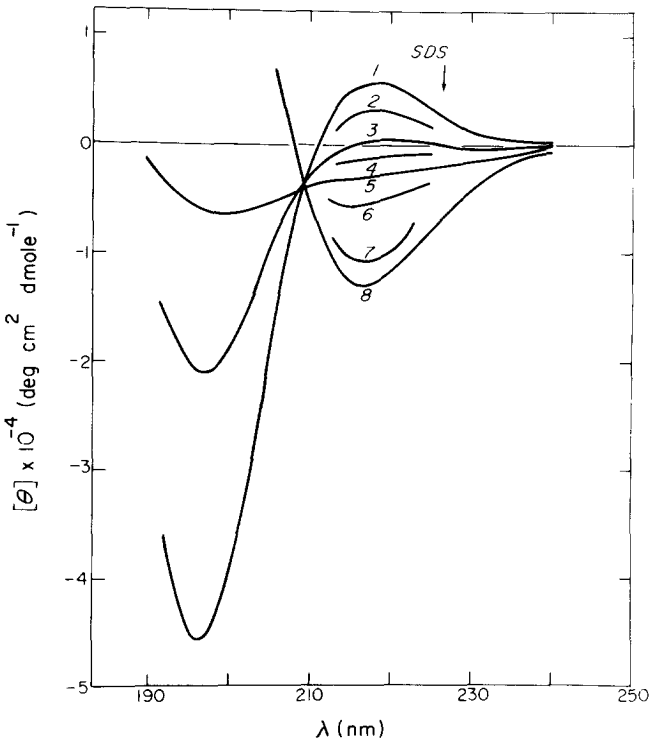

Figure 3. The CD of poly(L-lysine) in sodium dodecyl sulfate solutions at 25°C. PLL: 1.17×10^{-4} M. SDS: 1, 0 M; 2, 2.55×10^{-5} M; 3, 5.11×10^{-5} M; 4, 7.02×10^{-5} M; 5, 8.94×10^{-5} M; 6, 1.14×10^{-4} M; 7, 1.70×10^{-4} M; 8, 2.55×10^{-2} M.

residue), as judged from potentiometric measurements (unpublished data). The complexes redissolved at higher SDS concentrations above CMC and the CD spectra for both PLO and PLL were unaffected by the presence of excess surfactant micelles, suggesting a saturation of the polypeptide molecules with bound surfactant ions. Above CMC these micelles would merely solubilize the polypeptide-surfactant complexes.

That the induced changes in the polypeptide conformation by SDS occurs at very low SDS concentrations suggests a strong binding of these ions to the positively charged side groups of the polypeptides. However, the conformational transitions of the polypeptides cannot be explained on the basis of electrostatic interaction alone. It is well known that removal of the protons from the $-NH_3^+$ groups in PLL or hydrogen ion binding to the

Table I. Molar Residue Ellipticities of PLO and PLL in Surfactant Solutions[a]

	Surfactant	Temp. (°C)	Maximum λ (nm)	Maximum [θ][b]	Minimum λ (nm)	Minimum -[θ][c]	Minimum λ (nm)	Minimum -[θ][c]	Cross-over (nm)
Poly(L-ornithine)	SHS (C_{16})	45[d]	191.5	81000	209	31000±1200	222	32600±1000	202.5
	STS (C_{14})	35[d]	191.5	77000	209	30600±1600	222	32900±2200	202
	SDS (C_{12})	25	191.5	72000	209	25800±1400	222	28600±300	202
	SDeS (C_{10})	25	198	67000	210 Shoulder	4200±800	226	26100±600	208
	SOS (C_8)	25	195	58000	210 Shoulder	9700±1500	225	24400±1600	206
Poly(L-lysine)	SHS (C_{16})	45[d]	195	45000	217	15400±300			208
	STS (C_{14})	35[d]	195	41000	217	14400±200			208
	SDS (C_{12})	25	195	40000	217	13200±200			208
	SDeS (C_{10})	25	197	32000	219	11000±500			208
	SOS (C_8)	25	192.5	55000	210 Shoulder	15900±1300	225	28300±900	205

[a] Concentrations of polypeptides: 5×10^{-5} to 5×10^{-4} M (residue). Concentrations of surfactants: SHS, (1-7) $\times 10^{-2}$ M; STS, (1-10) $\times 10^{-2}$ M; SDS, (0.7-5) $\times 10^{-2}$ M; SDeS, (0.5-6) $\times 10^{-1}$ M; SOS, (6-10) $\times 10^{-1}$ M.

[b] Averages of duplicate experiments.

[c] Averages of at least three experiments.

[d] SHS and STS are sparingly soluble in water at room temperature.

$-COO^-$ groups of poly(L-glutamic acid) in aqueous solution induces a coil-to-helix transition, but uncharged PLO under the same conditions is only partially helical (ca. 20% (8,9)). In contrast the PLO-SDS complex is almost completely helical (Fig. 1). It seems that hydrophobic interactions among the alkyl chains of the surfactants would enhance the stability of the helical conformation.

Experimental. Hydrobromides of PLO (M.W. = 200000) and PLL (M.W. = 125000)(Pilot Chemicals) were converted to hydrochlorides through dialysis against 0.1 M HCl and then water. The polypeptide concentrations were determined by Kjeldahl nitrogen analysis. Surfactants were synthesized from pure alkyl alcohols (octyl and decyl from Aldrich Chemical Co.; dodecyl, tetradecyl and hexadecyl from Tokyo Kasei Co.) that had been further purified by vacuum distillation.

The CD spectra were measured with a Jasco J-10 under constant nitrogen flush. The data were expressed in terms of molar residue ellipticity in deg. cm^2 $dmole^{-1}$.

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