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Rep. Fac. Sci., Kagoshima Univ.
No. 32, 33~41, 1999

Potentiometric and Spectroscopic Characterization of Cationic Copolypeptide-Anionic Surfactant Complexes Formed by a Cooperative Binding System

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(Received August 10, 1999)

Keywords : copolypeptide-surfactant complex; cooperative binding; hydrophobic interaction; binding isotherm; FT-IR spectroscopy

Abstract

Binding behavior of an anionic surfactant, sodium dodecyl sulfate (SDS), to a series of L-lysine-containing copolypeptides in aqueous solutions was investigated in relation to the conformational change of copolypeptide-surfactant complexes, with the use of potentiometric and spectroscopic techniques. The present results of CD spectra and the binding isotherm of SDS by copolypeptides of opposite charge can lead us to conclude that SDS binds cooperatively to the positively charged side groups of a series of copolypeptides used in this work, resulting in the formation of a micelle-like cluster due to an additional hydrophobic interaction amoung bound SDS ions. Solid-state properties of the stoichiometric copolypeptide-SDS complexes were also examined by using CD and FT-IR spectroscopies; (Lys, Tyr) (1 : 1) and (4 : 1) systems adopt a β-pleated sheet conformation, while (Lys, Trp) (4 : 1) and (Lys, Phe)(1 : 1) systems adopt an α-helical conformation. Based on the results of FT-IR spectra, in all cases surfactant alkyl chains of SDS in the solid complexes were in an extended conformation.

Introduction

Conformational behavior is one of the important properties for proteins, because their functions are mainly linked to the dynamic change among different conformations *in vivo*. A number of homopolypeptides and copolypeptides have often been used as simple model compounds for the ordered conformations of complicated proteins. The interaction of a water-soluble homopolypeptide such as poly (L-lysine) ($(Lys)_n$) and an anionic surfactant such as sodium dodecyl sulfate (SDS) has been so far investigated in great detail (1-7). It is well known that SDS ions (DS^-s) bind cooperatively to positively charged $(Lys)_n$ by virtue of strong electrostatic and hydrophobic interactions and then induce conformational changes in polypeptides, far below the critical micelle concentration (cmc) (7-10). The cooperative binding of surfactant ions arises from hydrophobic interaction between the bound surfactant ions, and induces the formation of a micelle-like surfactant cluster and of the hydrophobic surroundings on the polypeptide. Copolypeptides such as poly (L-lysine, L-alanine) (11) and poly (L-lysine, L-serine) (12), which is a small step closer to real protein molecules, have been used as a better approach for the prediction of the secondary structure of proteins and the conformation of these copolypeptides has been investigated in detail.

Recently, the conformational and structural properties of the stoichiometric complexes formed by poly (L-glutamate) and oppositely charged surfactants have been investigated by Ponomarenko et al. (13, 14). They concluded, based on the results of X-ray diffractions and infrared spectra, that the polypeptide-surfactant complexes adopt lamellar

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structures consisting of alternating layers of polypeptide chains and bimolecular layers of surfactant, with the surfactant alkyl chains aligned perpendicular to the lamellar-surfaces and interdigitated.

Thus, the conformational and structural properties of model peptides in aqueous surfactant solutions and in the solid state are relevant to our understanding of the effects of surface active agents on protein structure. In addition, an understanding of these systems is also of fundamental importance in many biological processes and systems, including biomembranes, vesicles and the binding of small molecules to biopolymers.

In this paper, we studied the binding behavior of DS^- to a series of copolypeptides carrying L-lysine (Lys) and an aromatic amino acid such as L-tyrosine (Tyr), L-tryptophan (Trp) or L-phenylalanine (Phe) in aqueous solutions, by using a potentiometric technique and spectroscopic methods such as circular dichroism (CD), absorption and Fourier transform infrared (FT-IR) spectroscopies. In addition, the conformational and structural properties of copolypeptide-SDS complex in the solid state were also investigated. These results obtained for a series of copolypeptides were compared with that obtained for homopolypeptide, poly (L-lysine) ($(Lys)_n$) in relation to the conformation of copolypeptide-SDS complexes.

Experimental

Materials

All polypeptides used in this study were purchased from Sigma Chemical Co. and used without further purification; their molecular weights and degrees of polymerization are summarized in Table 1. A hydrobromide poly (L-lysine) $(Lys)_n$ and hydrobromide Lys-containing copolypeptides with a molar ratio of 4 : 1 or 1 : 1 were dissolved in 0.1 M (1 M = 1 mol dm^{-3}) hydrochloride and dialyzed against distilled water until no chloride was detected by silver nitrate. The concentration of stock solution of each copolypeptide containing an aromatic amino acid was determined from absorbance measurement of Tyr (λ_{max} = 275 nm, ε_{max} = 1340 $M^{-1}cm^{-1}$), Trp (λ_{max} = 278 nm, ε_{max} = 5500 $M^{-1}cm^{-1}$), and Phe (λ_{max} = 257 nm, ε_{max} = 190 $M^{-1}cm^{-1}$), respectively. The concentration of each copolypeptide, based on the mean residue of the aromatic amino acid, was fixed at 1.0×10^{-4} M throughout the experiment. The concentration of $(Lys)_n$ was determined by colloid titration with standard potasium poly (vinyl sulfate) solution. SDS was purified by recrystallization from ethanol three times; its critical micellar concentration (cmc) was determined to be 8.1×10^{-3} M. Laboratory deionized water was distilled twice. The pH of the sample solutions was not controlled but was found to be around 6.5.

Copolypeptide-SDS complex in the solid state was prepared by mixing equimolar quantities of copolypeptide and SDS solutions. The resulting white precipitate was isolated by centrifugation and washed three times with water to remove free surfactants and copolypeptides, and then dried in vacuum for at least 2 days.

Binding Isotherm Measurements

The binding isotherm of surfactant ion to oppositely charged polypeptide in aqueous solution was determined potentiometrically at 25℃, using a poly (vinyl chloride) (PVC) plastic membrane electrode which had been shown to respond to DS^- (15). This potentiometric technique is a powerful

TABLE 1. Molecular Weights and Degrees of Polymerization of Cationic Polypeptides Used in This Work

Polypeptide	Molar Ratio	Abbreviation	Molecular Weight	Degree of Polymerization
poly (L-lysine, L-tyrosine)	(1:1)	(Lys, Tyr) (1:1)	90000	440
poly (L-lysine, L-tyrosine)	(4:1)	(Lys, Tyr) (4:1)	24000	120
poly (L-lysine, L-tryptophan)	(4:1)	(Lys, Trp) (4:1)	38000	187
poly (L-lysine, L-phenylalanine)	(1:1)	(Lys, Phe) (1:1)	50000	281
poly (L-lysine)		$(Lys)_n$	26500	127

tool for the purpose of investigating the binding processes of DS^- to various cationic polypeptides in aqueous solution. The surfactant-selective membrane electrodes used in this work were fabricated using procedures which have been described by Satake et al. (9, 16) and Hayakawa and Kwak (17). The cell constructed was as in the following,

> Reference electrode (Ag-AgCl) | 3.3 M KCl Agar bridge | Reference solution (SDS, 1 mM) | PVC membrane | Sample solution | 3.3 M KCl Agar bridge | Reference electrode (Ag-AgCl).

The plastic PVC membrane electrode was prepared by dissolving 0.6 g of PVC and 1 mg of carrier complex composed of dimethyl dioctadecylammonium chloride and SDS into mixed solution of 2.4 ml of tricresyl phosphate and 15 ml of tetrahydrofuran. The solution was poured onto a covered glass dish and allowed tetrahydrofuran to evaporate slowly. A resulting film 0.30 mm thick was fixed to a PVC tube of 10 mm diameter with the use of tetrahydrofuran solution. The electromotive force (E) of the cell was measured with a modified Denki Kagaku Keiki Co., Ltd. Model COM8 pH meter with an accuracy of ± 0.2 mV. Prior to the measurments on copolypeptide-containing sample solutions, the PVC plastic membrane was calibrated with solutions of SDS. It was checked that this PVC membrane electrode excellently responds to DS^- in the concentration range studied.

Spectroscopic Measurements

Circular dichroim (CD) spectra were measured with a JASCO J-720 spectropolarimeter under the same experimental conditions as for binding isotherms and digitized data were transferred to a microcomputer and processed. CD spectra of the copolypeptide-SDS complex in the solid state were measured by casting from chloroform solution used as solvent on a quartz plate. Absorption spectra were recorded on a Shimadzu MPS-2000 spectrophotometer using 5 mm path length cell equipped with water-circulating jacket. FT-IR spectra of copolypeptide-SDS complexes in aqueous solution and in the solid state were measured with a Perkin-Elmer 1725X FT-IR spectrophotometer and an optical CaF_2 window. All spectroscopic measurements were made at 25℃ for aerated solutions.

Results and discussion

Binding Isotherm of SDS to Copolypeptide in Aqueous Solution

We examined the interaction between a series of copolypeptides (see Table 1) and SDS potentiometrically using surfactant-selective

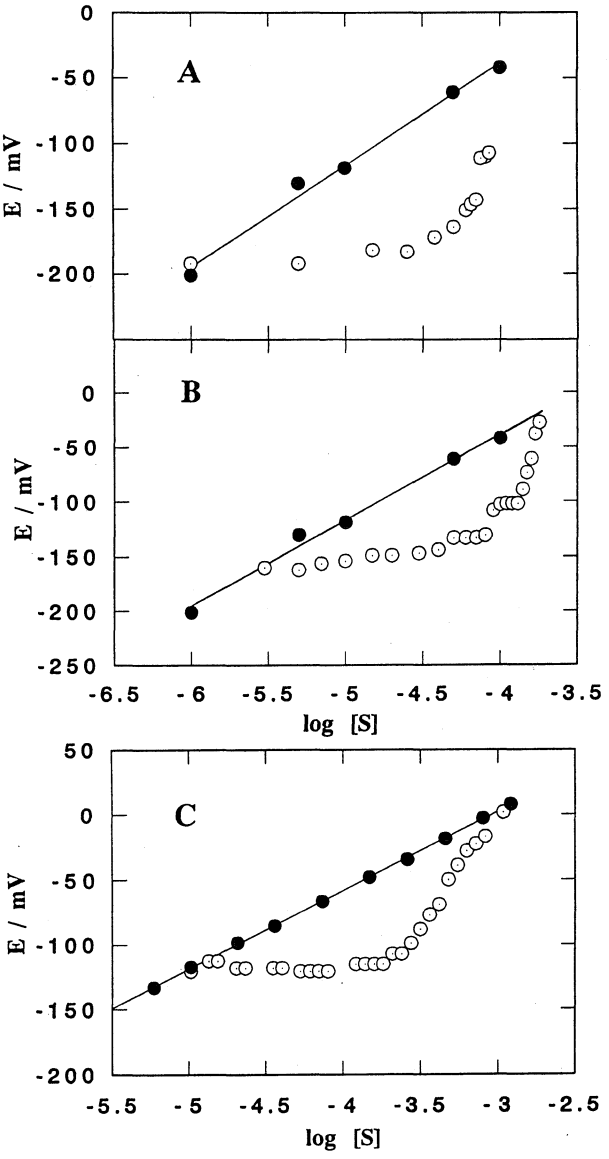

Fig. 1. The semilogarithmic plots of electromotive force (E) against SDS concentration for (A) (Lys,Tyr) (1:1), (B) (Lys, Tyr) (4:1) and (C) (Lys, Trp) (4:1) systems.

membrane electrodes. Figure 1 shows typical semilogarithmic plots of electromotive force (E) against SDS concentration ([S]) for (Lys, Tyr) (1:1), (Lys, Tyr) (4:1) and (Lys, Trp) (4:1) systems. The straight calibration line in the absence of copolypeptide (full circles in Fig. 1) indicates excellent performance with nearly Nernstian response over the concentration of SDS in this work. Upon addition of copolypeptide the observed E deviates from the calibration curve with an increase in [S], as shown by the open circles in Fig. 1. The first addition of SDS does not lead to binding, and all surfactant remains free (open circles overlap with calibration curve in Fig. 1). At a certain value of [S] binding of dodecyl sulfate ion (DS^-) occurs and free SDS concentration ($[S]_f$) remains nealy constant. By comparison with the calibration curve, $[S]_f$ is obtained and the degree of binding (β) of DS^- to the copolypeptide can be estimated by using a following relation.

$$\beta = ([S]-[S]_f) / [P] = [S]_b / [P] \qquad [1]$$

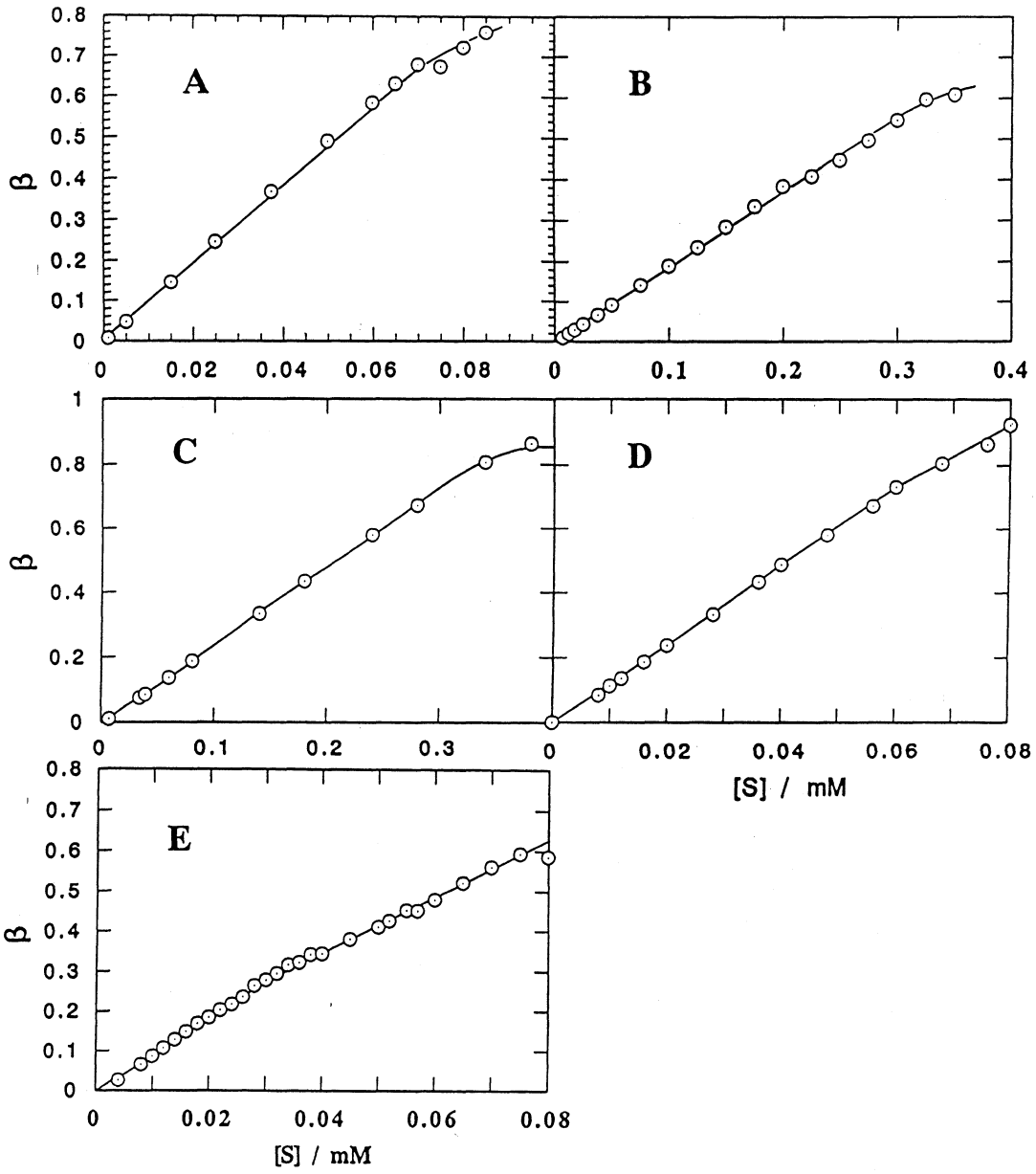

Fig. 2. Plots of β vs. SDS concentration ([SDS]) for (A) $(Lys)_n$, (B) (Lys, Tyr) (1:1), (C) (Lys, Tyr) (4:1), (D) (Lys, Trp) (4:1) and (E) (Lys, Phe) (1:1) systems.

where [S] is the total concentration of SDS, $[S]_f$ the equilibrium concentration of free (unbound) SDS, $[S]_b$ the concentration of bound DS^-, and [P] the residual concentration of copolypeptide ([P]=1 x 10^{-4} residue M). In Fig. 2, β of DS^- to the copolypeptide in aqueous solution obtained from Fig. 1 is plotted against [S] for all the systems. In all systems, β first increases almost linearly with increasing [S]. This result indicates that since the amino groups of Lys in the copolypeptide are almost completely ionized at pH 6.5, DS^- can interact electrostatically with amino groups of Lys, giving rise to an ion pair between them.

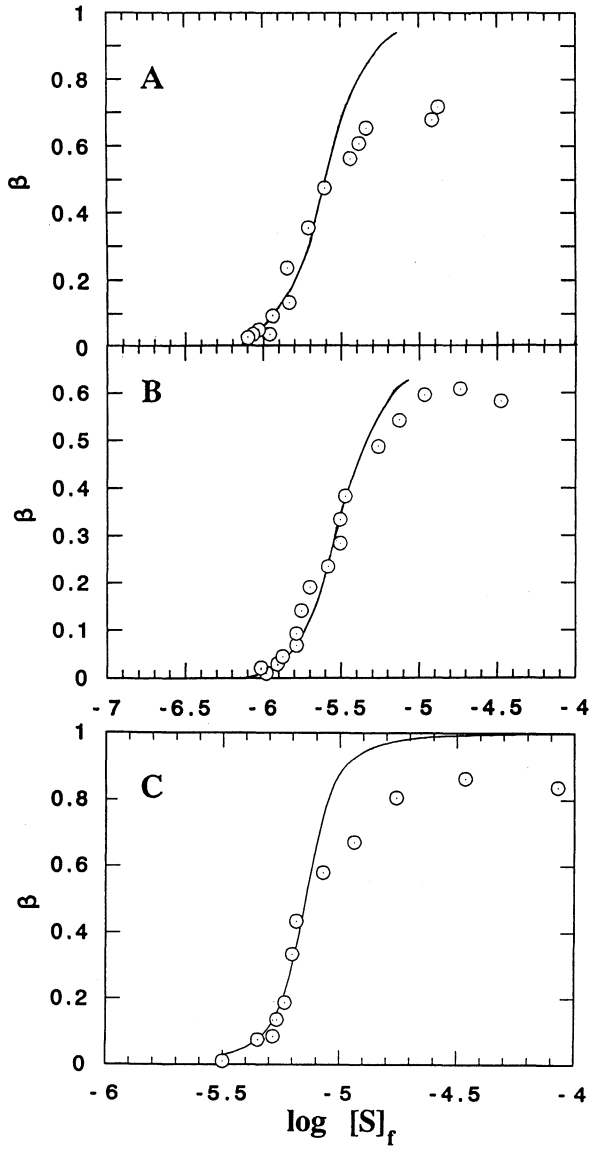

Fig. 3. Binding isotherms for (A) (Lys, Tyr) (1:1), (B) (Lys, Tyr) (4:1) and (C) (Lys, Trp) (4:1) systems. solid curve: calculatd curve from Eq. [2] with the values of u in Table 2 and [copolypeptide]=1 x 10^{-4} residue M.

Plots of β vs. $\log [S]_f$, i.e., the binding isotherms of DS^- to the copolypeptides in aqueous solutions are shown in Fig. 3 for the (Lys, Tyr) (1:1) and (4:1), and (Lys, Trp) (4:1) systems. In all cases the resulting binding isotherm showed a steep rise at a critical binding concentration; this result suggests that a cooperative binding of DS^- to the copolypeptide occurs in the range of SDS concentration far below the cmc, which was determined to be 8.1 x 10^{-3} M.

As was pointed out by Satake and Yang (8), the binding process of surfactant ion to polypeptide ion becomes cooperative because of an additional hydrophobic interaction among bound surfactant ions. In these circumstances, the bound surfactant ions cluster side by side onto polypeptide chain even in small degree of binding and cause the binding isotherm to rise steeply in the narrow region of $[S]_f$. β of DS^- to the polypeptide based on the theory of Satake and Yang (8) can be expressed in the following relation.

$$2\beta - 1 = (uK[S]_f - 1) / \{(1 - uK[S]_f)^2 + 4K[S]_f\}^{1/2} \quad [2]$$

where u is a cooperative parameter and measures the cooperativity of surfactant (SDS) binding: $u>1$ for cooperative binding, $u=1$ for noncooperative binding and $u<1$ for anticooperative binding. K is the intrinsic binding constant between a surfactant and an isolated polypeptide ion site. We can characterize the copolypeptide-surfactant interaction by a cooperative parameter (u); this parameter is determined by hydrophobic interaction among the bound surfactant ions. The solid line in Fig. 3 is the best fit of the experimental data at lower β to Eq. [2]. The binding isotherms for the (Lys, Tyr) (1:1) and (4:1) systems rise at $[S]_f = 1 \times 10^{-6}$ M, while in the case of (Lys, Trp) (4:1) system that rises at $[S]_f = 3.2 \times 10^{-6}$ M. The deviation at higher degrees of binding shown in Fig. 3 is attributed in part to the lack of an electrostatic term in Eq. [2]. The parameters u and K obtained for the best fit shown in Fig. 3 are listed in Table 2 for all the systems studied in this work. The

values of u estimated for the present systems reflect the cooperativity nature of the binding process. The cooperative nature of Lys-containing copolypeptide systems used in this work is considerably small compared to that for the $(Lys)_n$ system. The value of u for the (Lys, Tyr) (1:1) system is 9 and very small compared to those for other copolypeptide systems. As reported by Kurawaki et al. (18), the value of u obtained for poly (L-ornithine, L-tyrosine) (4:1) was determined to be 15 and smaller than that for (Lys, Tyr)(4:1) (u=30) . This may reasonably be ascribed to a change in hydrophobic interaction between neighboring groups. Since the methylene group of Lys side chain is longer than that of L-ornithine by one, the difference of u values obtained for (Lys, Tyr) (4:1) and poly (L-ornithine, L-tyrosine) (4:1) may reflect the difference of degree of hydrophobic interaction. It has been also pointed out that the value of u corresponds to an interchange energy containing an interaction energy between the binding sites (8, 9); its energy at 25℃ can be calculated to be 5.4 for (Lys, Tyr) (1:1), 8.4 for (Lys, Tyr) (4:1), 9.4 for (Lys, Trp) (4:1), 8.4 for (Lys, Phe) (1:1), and 11.4 kJ for $(Lys)_n$, respectively.

In addition, the average cluster size (m) of the bound DS^-s, i.e., the average number of bound DS^-s which constitute an one-dimensional micellar cluster on the copolypeptide chain, is given as

$$m=2\beta(u-1)/\{[4(1-\beta)(u-1)+1]^{1/2}-1\} \quad [3]$$

In Table 3, the values of m determined by using Eq. [3] and the values of u in Table 2 are given as a function of b. It should be noted that the cluster size (m) of bound DS^-s increases with an increase of β irrespective of the kind of copolypeptide. For instance, the value of m at $\beta=0.7$ for the (Lys, Trp) (4:1) system is calculated to be 7.7.

Conformation of Copolypeptide in Aqueous SDS Solution

The CD spectroscopy provides information about the secondary structure of copolypeptide in aqueous solution; the ellipticities between 190 and 250 nm in CD spectrum have been shown to be a sensitive indicator of the conformations of α-helix, β-sheet, and random coil of copolypeptides as well as homopolypeptides (19). Kusumoto and Akase (20) have measured SDS-concentration dependence of CD spectra for (Lys, Tyr) (1:1) and (4:1) systems in aqueous SDS solutions and assigned their conformations to be β-sheet, as summarized in Table 2. In SDS-free solutions, all Lys-containing copolypeptides except for (Lys, Tyr) (1:1) showed a strong negative CD band around 195-197 nm and a small positive band around 217 nm, which is typical of a random coil or an unordered conformation. This could be attributed to electrostatic repulsion between positively-charged ε-ammonium groups of Lys residues. With increasing SDS concentration or the degree of binding (β), the conformational change from the random coil to α-helix for the (Lys, Trp) (4:1) and (Lys, Phe) (1:1) systems was observed and $(Lys)_n$, (Lys, Tyr) (1:1) and (Lys, Tyr) (4:1) systems were found to adopt β-sheet dominantly, as summarized in Table 2. It should be noted that the conformation of Lys-containing copolypeptides in SDS solution depends on the kind of aromatic amino acid contained in the copolypeptide.

Table 2. Conformation and Cooperative Binding Parameters u and K for Cationic Polypeptide-SDS Systems

Polypeptide	Conformation	u	K
(Lys, Tyr) (1:1)	β-sheet	9	40000
(Lys, Tyr) (4:1)	β-sheet	30	11400
(Lys, Trp) (4:1)	α-helix	45	3100
(Lys, Phe) (1:1)	α-helix	30	4700
$(Lys)_n$	β-sheet	102	1500

It is of interest to find an interrelation between the conformational change induced by DS^- binding and the degree of binding (β) or the average cluster size (m). As shown in Table 3, in the case of (Lys, Trp) (4:1) and (Lys, Phe) (1:1) systems adopting α-helical structures, the values of m at $\beta=0.7$ are 11.9 and 10.0, respectively and correspond to the number of residues induced in about three turns of α-helical structure. From these findings, we could

Table 3. The Calculated Values of Average Cluster Size (m) of Bound Surfactant Ions As a Function of β

β	0.1	0.2	0.3	0.4	0.5	0.6	0.7
(Lys, Tyr) (1:1)	1.6	2.2	2.7	3.3	4.0	4.9	6.3
(Lys, Tyr) (4:1)	2.4	3.4	4.3	5.3	6.5	8.0	10.0
(Lys, Trp) (4:1)	2.8	4.0	5.1	6.3	7.7	9.5	11.9
(Lys, Phe) (1:1)	2.4	3.4	4.3	5.3	6.5	8.0	10.0
$(Lys)_n$	4.0	5.7	7.3	9.1	11.1	13.6	17.1

find an interrelation of β or m and the conformational change induced by DS^- for the present systems.

Therefore, the present results of CD spectra and the potentiometric measurement can lead us to conclude that the DS^- binds cooperatively to the positively-charged amino group of Lys of copolypeptide to give rise to a micelle-like cluster due to an additional hydrophobic interaction among bound DS^-. Thus, the environment of copolypeptide-SDS complexes formed by virtue of electrostatic and hydrophobic interactions is considered to be very hydrophobic.

Conformation of Copolypeptide-SDS Complexes in the Solid State

The conformation of copolypeptides and surfactant chains in the solid complexes was determined by using CD and FT-IR spectroscopies. In Fig. 4 are shown typical CD spectra of copolypeptide-SDS complexes in the solid state for all the systems studied in this work, which were cast from chloroform on a quartz plate. The CD spectral patterns for $(Lys)_n$, (Lys, Tyr) (1:1) and (Lys, Tyr) (4:1) systems (Fig. 4 A) represent the formation of β-sheet conformation, while in the case of copolypeptide carrying L-Trp or L-Phe those showed a double minimum at 208 and 222 nm characteristic of α-helix conformation. The conformation of copolypeptide chains in the SDS complexes correspond to those in aqueous SDS solutions in Table 2. The secondary structure of a protein is determined by its primary structure; each amino acid residue has a structure-forming potential, and the conformation of segments of a protein molecule may be dictated by

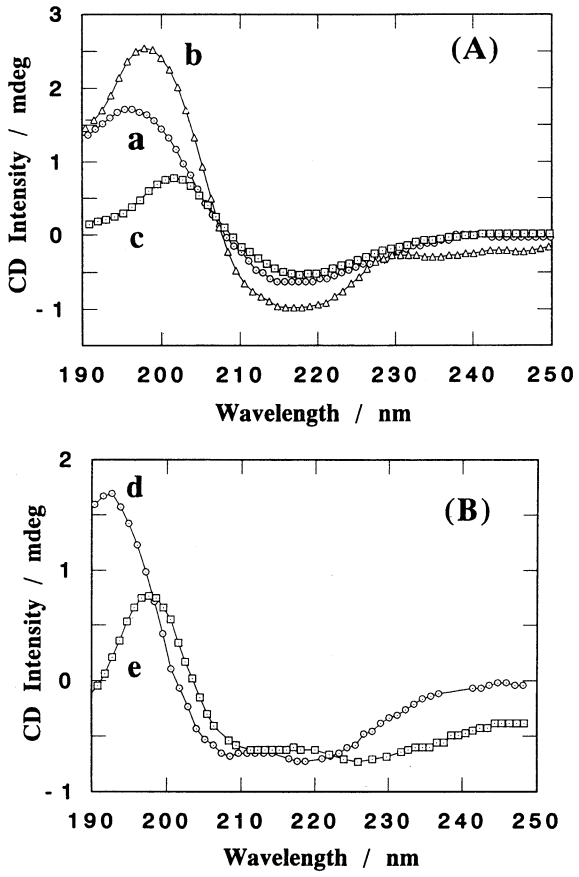

Fig. 4. CD spectra of copolypeptide-SDS complexes in the solid state. a) $(Lys)_n$, b) (Lys, Tyr) (1:1), c) (Lys, Tyr) (4:1), d) (Lys, Trp) (4:1), and e) (Lys, Phe) (1:1).

their average structure-forming potentials. Chou and Fasman (21) have computed the α-helix and β-sheet conformational parameters and formulated a set of empirical rules govering the folding of the secondary structural regions in proteins. According to them, Tyr is assigned to be an α-helix breaker and a β-sheet former, and Trp and Phe are α-helix formers. This fact is also reflected in the present systems and our CD spectral results correspond to the empirical rules proposed by Chou and Fasman . It can be, therefore, concluded that the conformation of copolypeptide-SDS complexes depends on the kind of aromatic amino acids contained in the copolypeptides used in this work.

FT-IR spectra also provide information about the conformation of the copolypeptide-surfactant complexes in aqueous solutions and in the solid state. In this work, we paid attention to C-H symmetric and

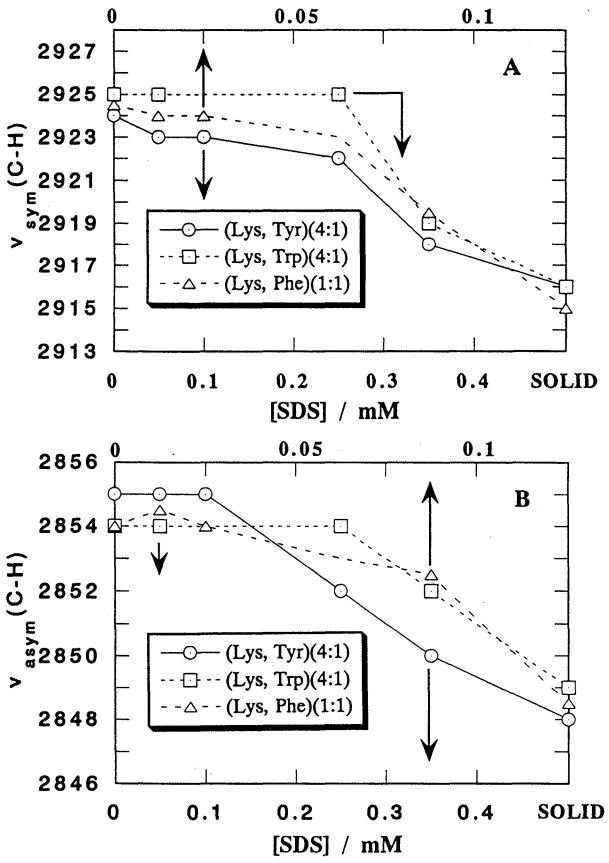

Fig. 5. IR frequencies of C-H symmetric (A) and C-H asymmetric (B) stretching vibrations of copolypeptide-SDS complexes in aqueous solution and in the solid state.

asymmetric vibrations of CH_3 group of the alkyl chains in aqueous SDS solutions and in the solid state. These vibrational bands were, respectively, observed at around 2925 and 2855 cm^{-1} in aqueous solution. SDS-concentration dependence of the frequencies of two vibrational bands for (Lys, Tyr) (4:1), (Lys, Trp) (4:1) and (Lys, Phe) (1:1) is shown in Fig. 5. The first addition of of SDS does not lead to shift of the frequencies. This indicates that transformation of surfactant chains is less extended and in a "liquid-like" state (22, 23). At [SDS]>0.25 mM for (Lys, Tyr) (4:1) and (Lys, Trp) (4:1) systems and [SDS]>0.06 mM for (Lys, Phe) (1:1) system, the frequencies of C-H stretching vibrations shift to lower frequencies indicating the transformation of SDS alkyl chains to adopt more extended conformation. The SDS concentration which induced the frequency shift observed for the present systems correspond to the degree of binding (β) of about 0.5, as can be seen from Fig. 2. Thus, it can be concluded that the transformation of SDS alkyl chains adopts transient conformation coexisting of less extended and extended ones, and finally converges to the "solid-like" state. Ponomarenko et al. (13, 14) have prepared stoichiometric complexes of poly (L-glutamate) and positively charged surfactants and discussed the structure of the solid complex with the use of infrared and X-ray diffraction methods. They concluded that the complexes are organized in lamellar structures consisting of alternating layers of poly (L-glutamte) chains separated by bimolecular layers of the surfactants and the surfactant alkyl chains are interdigitated and perpendicular to the lamellar surfaces. Similar structure for the present copolypeptide-SDS complexes in the solid state may be also adopted. A study of X-ray diffraction of the present systems is in progress.

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