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Interaction of Poly[*S*-(2-carboxyethyl)-L-cysteine] with Cationic Surfactants

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The induction of the β-structure of fully ionized poly[*S*-(2-carboxyethyl)-L-cysteine] in solution markedly depended on the kind of head group of cationic surfactants; it occurred by the addition of dodecylammonium chloride (DAC) but not by the addition of dodecyldimethylammonium chloride (DDAC) or dodecyltrimethylammonium chloride (DTAC), as concluded based on circular dichroism spectra. The induction of the β-structure by DAC was not inhibited by raising ionic strength of the medium but was enhanced when the polypeptide concentration was increased. The polypeptide took the β-structure in the precipitates produced by the addition of any one of these three surfactants, as indicated by the infrared absorption (IR) spectra. Intensity of a characteristic absorption band of carboxylate group around 1570 cm^{-1} for the complex with DDAC was vanishingly weak, while those with DAC and DTAC were medium and strong, respectively. The precipitates were solubilized in the presence of excess amount of surfactants. In the solubilized solution, the β-structure was again found in the case of DAC, irrespective of the polypeptide concentration, but it was found only at high polypeptide concentrations in the case of DDAC.

Interaction of anionic polypeptides with cationic surfactants has been examined by monitoring the secondary structure of polypeptides.[1-3] Recently, we have examined the effect of different cationic surfactants on the induction of the secondary structures of anionic polypeptides.[4,5] Three surfactants used in these studies were dodecylammonium chloride (DAC), dodecyldimethylammonium chloride (DDAC), and dodecyltrimethylammonium chloride (DTAC). They have different ionic heads but a common hydrocarbon tail (dodecyl group).

Induction of the α-helix of poly(L-glutamic acid) (PGA) occurred by the addition of these three surfactants.[4] On the other hand, the β-structure of poly(*S*-carboxymethyl-L-cysteine) (poly[Cys(CH_2COOH)]) was induced by the addition of DAC but not by the addition of DDAC or DTAC.[5] PGA has been used as a standard model polypeptide for the α-helix.[6-10] Characterization of the β-structure of poly[Cys(CH_2COOH)] has been carried out recently.[11-13]

The observed marked difference between PGA and poly[Cys(CH_2COOH)] is expected to originate from different nature of the two secondary structures; α-helix and β-structure. Direct interaction sites in either polypeptide with surfactant ammonium groups are carboxylate groups. In the complexes of poly[Cys(CH_2COOH)] with DDAC or DAC, proton transfer to carboxylate groups from the ammonium head groups was indicated in the solid state.[5] The extent of proton transfer depends on the intrinsic dissociation constant K_0 of carboxyl group for a given surfactant head group. There is a great difference between the values of pK_0 of the two polypeptides; pK_0=4.4 for PGA[8] and pK_0=3.2 for poly[Cys(CH_2COOH)].[14] It is possible, therefore, that different nature of the interactions of these two polypeptides with the same surfactants partly originates from a consequence of the different values of pK_0. In the present study, the interaction of poly-[*S*-(2-carboxyethyl)-L-cysteine] (poly[Cys(CH_2CH_2COOH)]) with the three same cationic surfactants, DAC, DDAC, and DTAC, is examined by observing circular dichroism of solutions and infrared absorption spectra of precipitates. This polypeptide is a side-chain homolog of poly[Cys(CH_2COOH)] and carries carboxyl groups of pK_0=4.0.[14] Consequences of different values of pK_0 of carboxyl groups and different numbers of methylene groups are expected to show up in the interaction with the surfactants.

Experimental

Poly[*S*-(2-carboxyethyl)-L-cysteine] (poly[Cys(CH_2CH_2COOH)]) was synthesized as described previously[15] and its weight-average molecular weight and degree of polymerization were 3.5×10^4 and 200, respectively, as estimated from the viscosity-molecular weight relation on poly[Cys(CH_2-$COOH$)].[16] Three surfactants used in the present study were the same lots as used in the previous studies.[4,5] Values of critical micelle concentration (cmc) in 0.01 M† NaCl are 1.23×10^{-2} M[17] and 1.14×10^{-2} M[18] for DAC and DDAC, respectively. Values of cmc for DTAC are 2.16×10^{-2} M (in water) and 1.59×10^{-2} M (in 0.02 M NaCl).[17] Concentrations of polypeptide C_p and surfactant C_D are expressed in residue molarity and molarity, mol dm^{-3} (M), respectively. Composition of the solutions is specified by C_p and the mixing ratio C_D/C_p. Circular dichroism (CD) spectra were taken on a Jasco J 40A circular dichrograph using cells of light paths of 1 and 10 mm. Four scans were averaged in most cases. Infrared absorption (IR) spectra of polypeptide-surfactant complexes in KBr discs were obtained on a Jasco IRA-2 spectrophotometer. Measurements of CD and IR were carried out at 24±2 °C.

Effects of different ways of preparing solutions were examined previously[5] and they were shown not to affect the obtained results significantly in so far as measurements were made on the solutions incubated more than 12 h. In

† 1 M=1 mol dm^{-3}.

the present study, solutions were prepared by adding small volumes of concentrated surfactant stock solutions to polypeptide solutions and incubated at 24±2 °C for 12 to 24 h.

Results

Interactions in 1×10^{-2} M Tris HCl (pH 7.4) in the absence of other supporting electrolytes were examined in the first place. In Fig. 1, CD spectra of solutions at a residue concentration C_p of 1×10^{-4} M are shown. In the absence of surfactants, the CD spectra (labeled a in Fig. 1A—C) of random coils are characterized by two negative bands, one around 200 nm and the other at 225 nm. On addition of DAC, the residue ellipticity, $[\theta]$, around 200 nm increased and became positive, while that at 225 nm became more negative. At the mixing ratio of 2.2, the spectrum (e) resembles that of typical β-structure (although incomplete) induced by protonation. On the other hand, no significant change occurred on addition of DDAC or DTAC. Effects of three surfactants can be compared clearly when values of residue ellipticities around band positions, $[\theta]_{205}$ and $[\theta]_{225}$, are plotted against the mixing ratio C_D/C_p (Fig. 2). The β-structure of poly[Cys(CH_2CH_2COOH)] was induced by the addition of DAC but not by DDAC or DTAC, similar to the previous result on poly[Cys(CH_2COOH)].[5] Further addition of the surfactants caused precipitation of the polypeptide. Precipitation occurred at the mixing ratios of about 3 for DAC, 15 for DDAC, and 20 for DTAC. When the mixing ratios exceeded 100, however, solubilization took place and the conformation of the polypeptide in the solubilized solutions was again the β structure in the case of DAC but random coils in the case of DDAC or DTAC.

The results at $C_p=1\times10^{-3}$ M are shown in Fig. 3. Induction of the β-structure by DAC was significantly enhanced when the polypeptide concentration was raised. Induction of the β-structure did not take place in the case of DDAC or DTAC even at this concentration. Precipitation occurred at the mixing ratios considerably smaller than those at $C_p=1\times10^{-4}$ M: about 0.8 for DAC, 1 for DDAC, and 3 for DTAC. Solubilization occurred for DAC when mixing ratios exceeded 100 and for DDAC ($C_D/C_p>400$) but not for DTAC up to $C_D/C_p=500$. In solubilized solutions, the β-structure was found for DAC and also for DDAC, although the extent of induction was not large for the latter.

Interaction in the presence of 0.1 M NaCl was examined at $C_p=1\times10^{-4}$ M (Fig. 4). As compared with the result on DAC in the absence of NaCl, increase in ionic strength slightly inhibited the induction of the β-structure by DAC. No induction by DDAC or DTAC was also found in the presence of 0.1 M NaCl. Solubility of the polypeptide decreased in 0.1 M NaCl, since precipitation of the polypeptide occurred at the mixing ratios (about 2, 6, and 12 for DAC, DDAC, and DTAC, respectively), which were smaller than those found in the absence of 0.1 M NaCl. Solubilization at high mixing ratios occurred only for DAC.

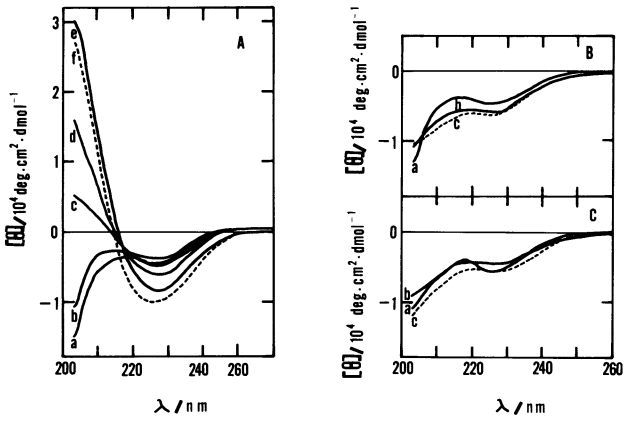

Fig. 1. CD spectra of poly[Cys(CH_2CH_2COOH)] in 1×10^{-2} M Tris HCl (pH 7.4) at $C_p=1\times10^{-4}$ M. (A) DAC. Mixing ratios (C_D/C_p): (a) 0, (b) 0.6, (c) 1.0, (d) 1.4, (e) 2.2, and (f) 100. (B) DDAC. Mixing ratios (C_D/C_P): (a) 0, (b) 6.2, and (c) 150. (C) DTAC. Mixing ratios (C_D/C_p): (a) 0, (b) 14, and (c) 100.

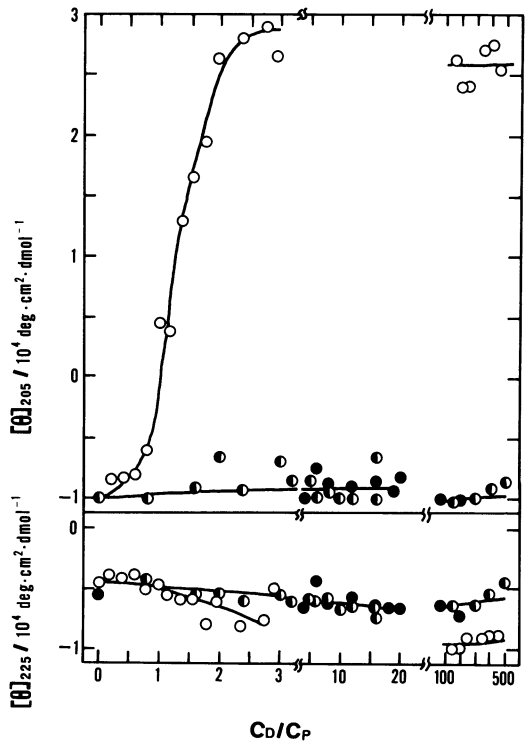

Fig. 2. Dependence of residue ellipticities $[\theta]_{205}$ and $[\theta]_{225}$ on the mixing ratio C_D/C_p in 1×10^{-2} M Tris HCl (pH 7.4) at $C_p=1\times10^{-4}$ M. (○) DAC, (◐) DDAC, and (●) DTAC.

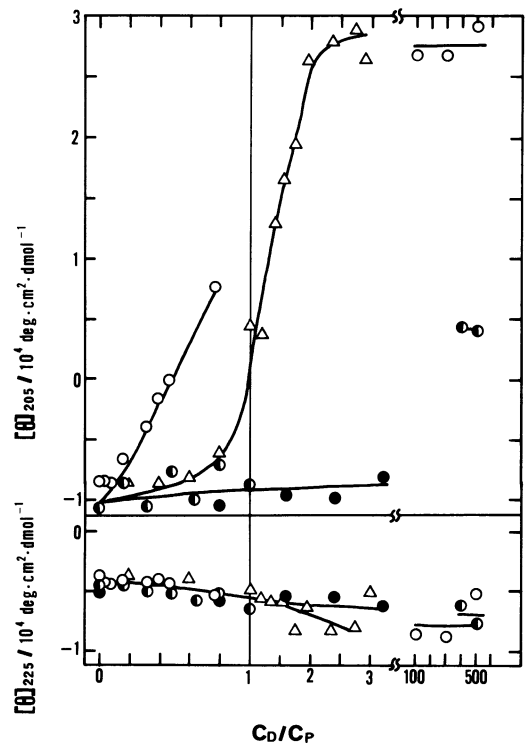

Fig. 3. Dependence of residue ellipticities $[\theta]_{205}$ and $[\theta]_{225}$ on the mixing ratio C_D/C_p in 1×10^{-2} M Tris HCl(pH 7.4) at $C_p=1\times10^{-3}$ M. (○) DAC, (◐) DDAC, and (●) DTAC. Data at $C_p=1\times10^{-4}$ M (△) in the case of DAC are also shown for comparison.

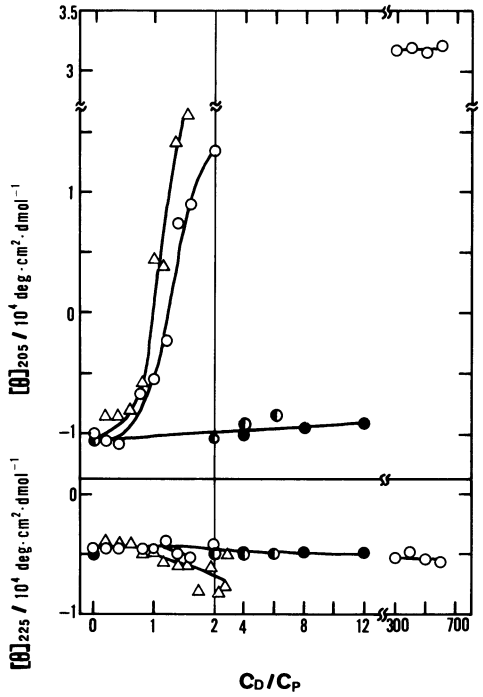

Fig. 4. Dependence of residue ellipticities $[\theta]_{205}$ and $[\theta]_{225}$ on the mixing ratio C_D/C_p in 1×10^{-2} M Tris HCl (pH 7.4) in 0.1 M NaCl at $C_p=1\times10^{-4}$ M. (○) DAC, (◐) DDAC, and (●) DTAC. Data for DAC in the absence of 0.1 M NaCl (△) are also shown for comparison.

In Fig. 5, correlation between two residue ellipticities of the polypeptide at 205 nm and 225 nm is shown. The data in the presence of DAC (open symbols) show two distinct regions; one corresponds to the induction of the β-structure at low mixing ratios and the other to solubilized solutions. The magnitude of the negative band around 225 nm, which is associated with n-π* transition of the peptide chromophore, is greater in the solubilized solutions than in the solutions at low mixing ratios. This increased magnitude represents a decrease of the polarity around peptide chromophore in the solubilized state. This environmental effect on $[\theta]_{225}$ is very weak in solutions containing DTAC (filled symbols). In the presence of DDAC (half-filled symbols), however, the magnitude of $[\theta]_{225}$ increases considerably even for random coils at $C_p=1\times10^{-4}$ M, which is likely a preinduction stage of the β-structure at $C_p=1\times10^{-3}$ M in the solubilized solutions. Precipitation, solubilization and the conformation of the polypeptide in solution are summarized in Fig. 6. Three zones (A, B, C) for each surfactant represent, respectively, the results obtained at $C_p=1\times10^{-4}$ M, at $C_p=1\times10^{-3}$ M, and at $C_p=1\times10^{-4}$ M in 0.1 M NaCl. It is to be noted that 1×10^{-2} M Tris buffer (pH=7.4) was present in every case.

Infrared absorption (IR) spectra (Figure 7) of the complexes of poly[Cys(CH_2CH_2COOH)] with three surfactants were taken on the precipitates obtained at

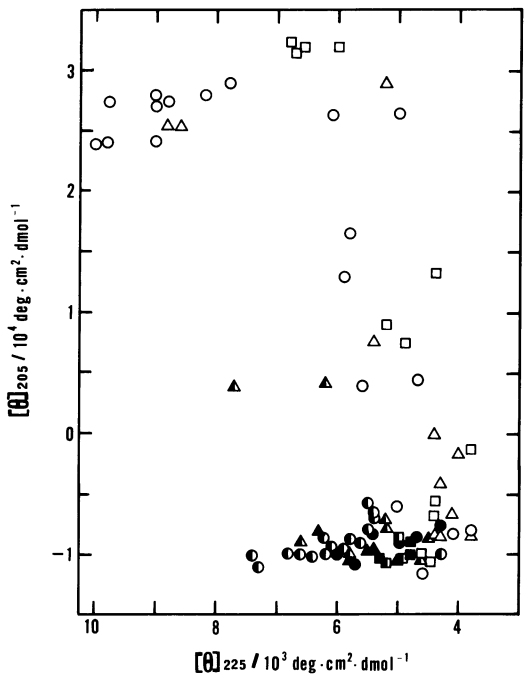

Fig. 5. Correlation between $[\theta]_{205}$ and $[\theta]_{225}$. Open, half-filled, and filled symbols represent the data on DAC, DDAC, and DTAC, respectively. Circles; $C_p=1\times10^{-4}$ M. Triangles; $C_p=1\times10^{-3}$ M. Squares; $C_p=1\times10^{-4}$ M in 0.1 M NaCl.

C_p=1×10^{-2} M and C_D/C_p=1. Amide I bands were observed at 1630 cm^{-1} [$\nu(\pi,0)$][19] and 1695 cm^{-1} [$\nu(0,\pi)$][19] and amide II bands were observed at 1525 cm^{-1} for three complexes, and hence antiparallel β-structure was confirmed as the conformation of the polypeptide. Proton transfer from ammonium head group to carboxylate was judged by measuring the intensity of antisymmetric streching mode of COO$^-$ groups found at 1570 cm^{-1}. The intensity was very weak, medium, and strong in the complexes with DDAC, DAC, and DTAC, respectively, if compared with the intensity of amide II band in each case. Thus, protonation of carboxylate groups was indicated in the complexes with DDAC and DAC.

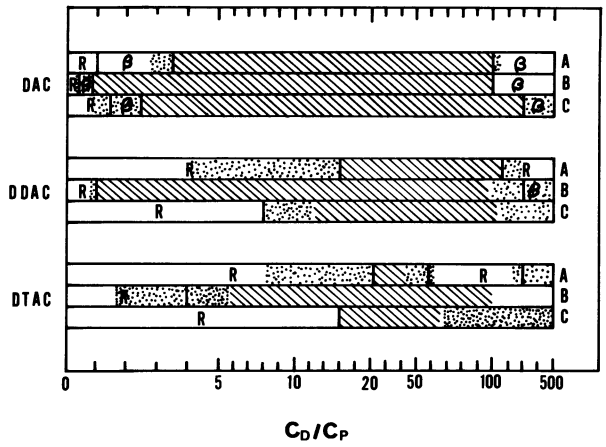

Fig. 6. Schematic phase diagram of a mixture of poly[Cys(CH_2CH_2COOH)] in the presence of a surfactant in 1×10^{-2} M Tris HCl (pH 7.4). Three zones A, B, and C correspond to the data at C_p=1×10^{-4} M, C_p=1×10^{-3} M, and C_p=1×10^{-4} M in 0.1 M NaCl, respectively. Blank region; solution. Dotted region; turbid solutions. Hatched region; precipitates. Conformations of the polypeptide in solutions are designated by R (random coils)or β(β-structure).

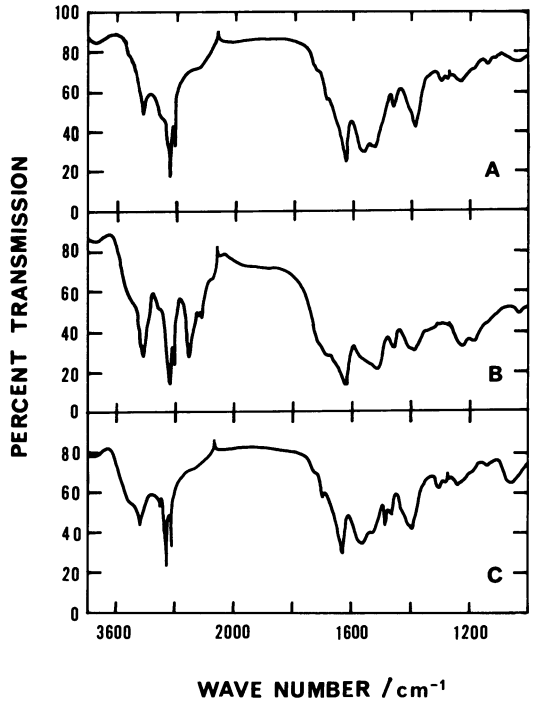

Fig. 7. Infrared absorption spectra of precipitates containing poly[Cys(CH_2CH_2COOH)] complexed with DAC (A), DDAC (B), and DTAC (C).

Discussion

Present results on poly[Cys(CH_2CH_2COOH)] and previous ones on poly[Cys(CH_2COOH)] are summarized in Table 1 and compared with each other. Very similar results are obtained between these two polypeptides with respect to the induction of the β-structure in solution as well as the effects of ionic strength and the polypeptide concentration on the induction. This comparison indicates that difference in acidities of the carboxyl groups between the two polypeptides scarcely affects their interaction with the cationic surfactants. It is concluded from this finding that the different interactions of the three surfactants with PGA[4] and poly[Cys(CH_2COOH)][5] essentially arise from different characters of the two secondary structures, α-helix and β-structure, and cannot be attributed much to different nature of the carboxyl groups of the two polypeptides. Therefore, present study confirms the validity of the previous discussion.[5]

On the other hand, there are several significant differences between the results on poly[Cys(CH_2COOH)] and poly[Cys(CH_2CH_2COOH)]. The addition of DDAC induced the β-structure of poly[Cys(CH_2CH_2COOH)] to some extent under a limited condition; solubilized solutions at high polypeptide concentrations. Induction of the β-structure of poly[Cys(CH_2COOH)] by the addition of DDAC did not occur under any condition examined. Conformations of the complexes with DTAC were random coil in the case of poly[Cys(CH_2COOH)] but the β-structure in the case of poly[Cys(CH_2CH_2COOH)]. Measurements of CD in solubilized solutions were hampered by the presence of (assumed) large aggregates in the case of the following combinations: DTAC–poly[Cys(CH_2CH_2-$COOH$)] and DDAC–poly[Cys(CH_2COOH)].

In addition to different values of pK_0 of side chain carboxyl groups, there is another important consequence of different side chains between these two polypeptides; different extents of hydrophobic nature of the side chains. Induction of the β-structure by DDAC in the solubilized solutions at C_p=1×10^{-3} M as well as the β-conformation of the complex with DTAC in the solid state can be ascribed to the enhanced stability of the β-structure of poly[Cys-(CH_2CH_2COOH)] due to increased nonpolar nature.[14,20]

Table 1. Comparison of Major Conformations of the Two Polypeptides in the Presence of Three Surfactants

	Solution		Precipitates		Solubilized solution	
Polypeptide[a]	M	E	M	E	M	E
DAC	beta	beta	beta	beta	beta	beta
DDAC	coil	coil	beta	beta	x[b]	beta (1 mM) coil (0.1 mM)
DTAC	coil	coil	coil	beta	coil	x[b] (1 mM) coil (0.1 mM)

a) Poly[Cys(CH_2COOH)] and poly[Cys(CH_2CH_2COOH)] are abbreviated as M and E, respectively. b) Conformations could not be assessed from highly perturbed CD spectra.

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