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# Effects of Cationic Surfactants on the Conformation of Poly[(*S*)-(carboxymethyl)-L-cysteine]

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ABSTRACT: The induction of the β-structure of fully neutralized poly[(*S*)-(carboxymethyl)-L-cysteine] in solution depends markedly on the head groups of cationic surfactants. It occurs only on the addition of dodecylammonium chloride (DAC) but not by the addition of dodecyldimethylammonium chloride (DDAC) or dodecyltrimethylammonium chloride (DTAC), as revealed by circular dichroism. Infrared absorption spectra of the precipitates produced by the addition of an excess amount of each of the three surfactants show that the polypeptide assumes the β-structure in the complexes with DAC or DDAC, but that the random coil conformation is dominant in the complex with DTAC. The intensity of a characteristic absorption band of the carboxylate group around 1585 $cm^{-1}$ in the complexes with surfactants is vanishingly weak for DDAC, weak for DAC, but very strong for DTAC. Decrease of pH in salt-free solutions occurs on addition of the surfactants in the order DAC > DDAC > DTAC. The effects of ionic strength and polypeptide concentration on the induction of the β-structure differ markedly from those found on the induction of the α-helix of poly(L-glutamic acid) by the same surfactants.

## Introduction

The interaction of ionic surfactants with ionic polypeptides has been studied extensively.[1-12] However, the effects of different head groups on the interaction have received less attention than those of different hydrocarbon chain lengths. It was found in a recent study that the α-helix of poly(L-glutamic acid) (PGA) was induced by three cationic surfactants that had different head groups but a common hydrocarbon tail.[13] Marked differences were observed among these three surfactants when they were added to an extent more than required to induce the α-helix, while a small difference was found among their inducing powers.

In the present study, the effects of the three surfactants used in the previous study,[13] dodecylammonium chloride (DAC), dodecyldimethylammonium chloride (DDAC), and dodecyltrimethylammonium chloride (DTAC), on the conformation of poly[(*S*)-(carboxymethyl)-L-cysteine] (poly[Cys($CH_2COOH$)]) are examined by circular dichroism (CD) in solution and by infrared absorption (IR) in the solid state. In the light of recent characterizations of the β-structure of poly[Cys($CH_2COOH$)],[14-16] the β-structure found in the present study is most likely (although not confirmed) to consist of aggregates of folded chains.

## Experimental Section

The weight-average molecular weight and the degree of polymerization of the sample of poly[Cys($CH_2COOH$)] used in the present study were $5.8 \times 10^4$ and 360, respectively. Some characterizations of the sample were given in a previous report.[16] The

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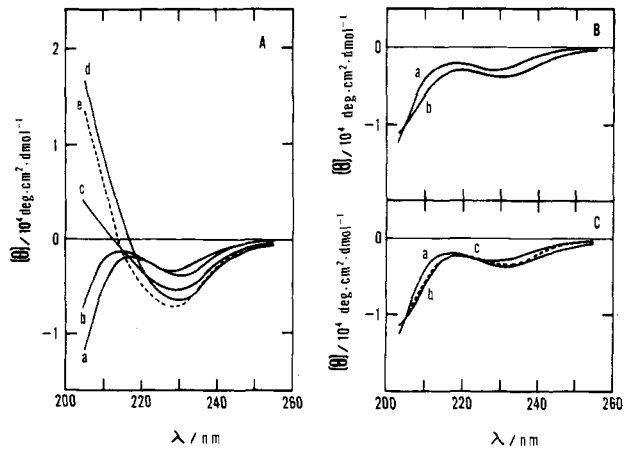


**Figure 1.** CD spectra of poly[Cys(CH$_2$COOH)] in the presence of various amounts of the three surfactants in $1 \times 10^{-2}$ M Tris buffer (pH 7.4) at $C_p = 1 \times 10^{-4}$ M. (A) DAC: $C_D/C_p = 0$ (a), 0.5 (b), 1.25 (c), 2.5 (d), and 300 (e, dashed curve). (B) DDAC: $C_D/C_p = 0$ (a) and 10 (b). (C) DTAC: $C_D/C_p = 0$ (a), 15 (b) and 125 (c, dashed curve).

three surfactants were of the same lot as used previously.[13]

Solutions were prepared in the following way unless otherwise stated. Polymer stock solutions (about $1 \times 10^{-2}$ residue mol/L (M)) were prepared by dissolving the sodium salt in water or $1 \times 10^{-2}$ M Tris·HCl (pH 7.4) (in the presence or absence of 0.1 M NaCl). Stock solutions of surfactants (about 0.1 M) were prepared only in water. Mixing was carried out by gradually adding a small volume of surfactant stock solution to a polymer solution of a desired concentration $C_p$ in a desired solvent. Addition of the surfactant solution did not significantly change the composition of the solvent and the polymer concentration. The surfactant concentration is denoted as $C_D$. Final solutions were incubated for about 24 h at ambient temperature (24 ± 2 °C) before measurements.

A different way of mixing was also examined in which a small volume of stock solution of surfactant was added quickly all at once to a polymer solution. Essentially identical results were obtained after the incubation time irrespective of the mixing procedure, unless the mixing ratio $C_D/C_p$ was large ($C_D/C_p > 100$). At mixing ratios greater than ~100, less turbid solutions were usually obtained by the latter mixing procedure than the former.

KBr disks for IR measurements were prepared as follows. Dry KBr powder was mixed with precipitates (dried), which were formed at $C_D/C_p = 1$ and $C_p = 1 \times 10^{-2}$ M in $1 \times 10^{-2}$ M Tris buffer (pH 7.4) and dried over $P_2O_5$ for several days. The mixture was finely ground and then kept in a desiccator over $P_2O_5$ for several more days. Afterward, it was pressed to a pellet under reduced pressure.

CD spectra were taken on a Jasco J-40 A circular dichrograph, using cells of 1- and 10-mm light paths. Four scans were averaged. Measurements of pH were carried out on an Iwaki M-225 pH meter. IR spectra were obtained with a Jasco IRA-2 spectrophotometer on KBr disks. These three measurements were made at 24 ± 2 °C.

Critical micelle concentrations (cmc) of the surfactants in $1 \times 10^{-2}$ M Tris buffer (pH 7.4) were not determined in the present study, since solubilities of deprotonated species were found to be very low for both DAC and DDAC. In $1 \times 10^{-2}$ M NaCl solutions, precipitation of DDAC occurred at pH 6.7 and 6.5 for $C_D$ of $5 \times 10^{-3}$ and $10 \times 10^{-3}$ M, respectively, and flocculation of DAC occurred at pH 8.8, 7.9, and 7.2 for $C_D$ of $1 \times 10^{-3}$, $5 \times 10^{-3}$ and $10 \times 10^{-3}$ M, respectively. In water, the values of cmc are $1.2 \times 10^{-2}$, $1.4 \times 10^{-2}$, and $1.9 \times 10^{-2}$ M for DAC, DDAC, and DTAC, respectively.[13]

## Results

**I. Interactions in $1 \times 10^{-2}$ M Tris Buffer (pH 7.4).** In Figure 1 are shown CD spectra of poly[Cys(CH$_2$COOH)] at $C_p = 1 \times 10^{-4}$ M in $1 \times 10^{-2}$ M Tris buffer (pH 7.4) in the presence of various amounts of each of the three surfactants. When the surfactants were not added, CD spectrum a was obtained, characterized by two negative bands, one around 200 nm and the other around 230 nm. This type of CD spectrum represents the charged random coil state of the polypeptide.[14-16] On addition of DAC, the CD spectra changed as shown in Figure 1A. The band around 200 nm became less negative and then became positive as the mixing ratio increased. At a mixing ratio of 2.5, the residue ellipticity at 205 nm ($[\theta]_{205}$) reached a value of $\sim 1.6 \times 10^4$. The other band around 230 nm became slightly more negative as $C_D/C_p$ increased. Spectrum d in Figure 1A resembles that of the β-structure of the polypeptide induced either by protonation[14,16] or by the binding of bivalent metal cations.[17] The peak of the CD band around 200 nm could not be reliably resolved due to the absorption of $Cl^-$ ions. Residue ellipticities at 205 and 230 nm are plotted against the mixing ratio for the three surfactants in Figure 2.

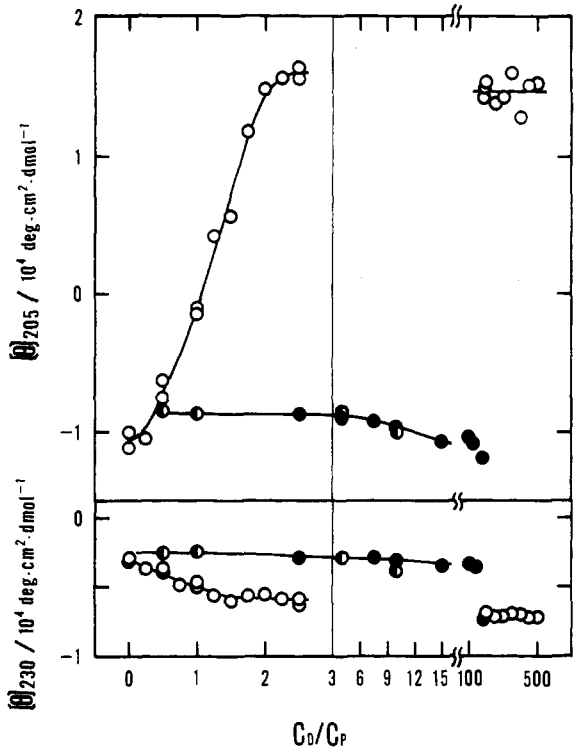


**Figure 2.** Dependence of $[\theta]_{205}$ and $[\theta]_{230}$ on the mixing ratio $C_D/C_p$ in $1 \times 10^{-2}$ M Tris buffer (pH 7.4) at $C_p = 1 \times 10^{-4}$ M: (○) DAC, (◐) DDAC, and (●) DTAC.

As indicated in Figures 1 and 2, the β-structure of poly[Cys(CH$_2$COOH)] is induced by the addition of DAC, while it is not induced in solution by the addition of DDAC or DTAC.

**Effects of Polypeptide Concentration.** The interaction with the surfactants was also examined at $C_p = 1 \times 10^{-3}$ M. In Figure 3, $[\theta]_{205}$ and $[\theta]_{230}$ are plotted against the mixing ratio $C_D/C_p$. The result at $C_p = 1 \times 10^{-4}$ M in the presence of DAC is also shown as triangles. The induction of the β-structure occurs at lower mixing ratios, and the extent of the induction is greater at $C_p = 1 \times 10^{-3}$ than at $1 \times 10^{-4}$ M. At $C_p = 1 \times 10^{-3}$ M, turbidity appeared for the range of the mixing ratio larger than the point indicated by a vertical line in Figure 3. The enhancement of the induction at high polymer concentrations indicates that the β-structure is stabilized partly by intermolecular association, consistent with the suggested picture in a recent study.[16] In the case of DDAC and DTAC, induction of the β-structure did not occur even at $C_p = 1 \times 10^{-3}$ M.

**Effects of Ionic Strength.** In Figure 4, $[\theta]_{205}$ and $[\theta]_{230}$ at $C_p = 1 \times 10^{-4}$ M in Tris buffer (pH 7.4) + 0.1 M NaCl

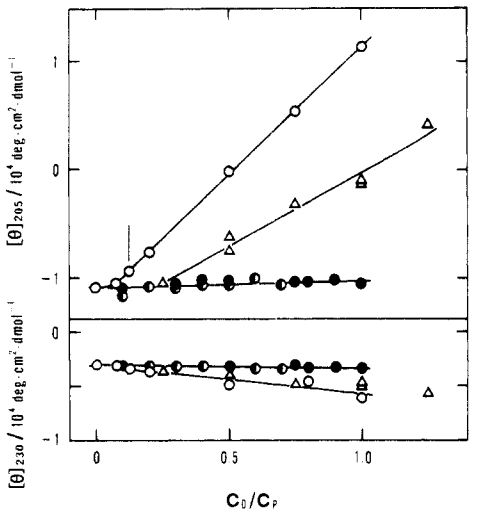


**Figure 3.** Dependence of $[\theta]_{205}$ and $[\theta]_{230}$ on the mixing ratio $C_D/C_p$ in $1 \times 10^{-2}$ M Tris buffer (pH 7.4) at $C_p = 1 \times 10^{-3}$ M: (○) DAC, (◐) DDAC, and (●) DTAC. The data for DAC at $C_p = 1 \times 10^{-4}$ M (△) are also shown. Turbidity appeared at the mixing ratio indicated by a vertical line.

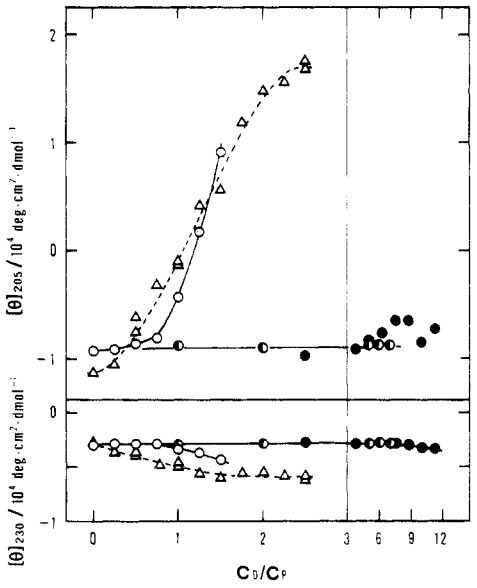


**Figure 4.** Dependence of $[\theta]_{205}$ and $[\theta]_{230}$ on the mixing ratio $C_D/C_p$ in $1 \times 10^{-2}$ M Tris buffer (pH 7.4) + 0.1 M NaCl at $C_p = 1 \times 10^{-4}$ M: (○) DAC, (◐) DDAC, and (●) DTAC. The data for DAC in the absence of 0.1 M NaCl (△) are shown by a dashed curve.

are plotted against the mixing ratio for the three surfactants. For comparison, the result in the absence of 0.1 M NaCl is also shown in the case of DAC by triangles (a dashed curve). In contrast to the induction of the $\alpha$-helix of PGA,[13] the addition of salt did not interfere with the interaction between DAC and poly[Cys($CH_2COOH$)]. It should be noted that the inhibitory action of increased ionic strength is also present in the DAC–poly[Cys($CH_2COOH$)] system in the range of the mixing ratio below about 1.0. However, another effect of ionic strength, probably the salting out of the $\beta$-structure that caused the aggregation and eventually precipitation, affords additional stability to the $\beta$-structure over random coils and favors its formation. In the case of DDAC and DTAC, addition of NaCl had almost no effect.

The effect of added salt found here is very similar to that of the interactions of poly[Cys($CH_2COOH$)][17] or its side-chain homologue[18] with bivalent metal cations. In other words, the addition of salt does not interfere with the

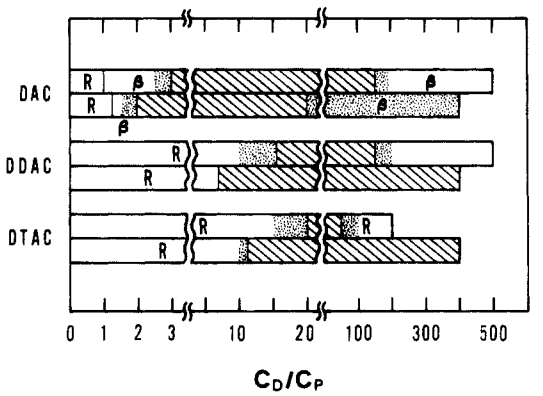


**Figure 5.** Dispersed states and the polypeptide conformation in $1 \times 10^{-2}$ M Tris buffer (pH 7.4) at $C_p = 1 \times 10^{-4}$ M in the absence (upper zones) and the presence (lower zones) of 0.1 M NaCl: blank region, clear solution; dotted region, turbid solution; shaded region, precipitation. R and $\beta$ denote the random coil and the $\beta$-structure, respectively.

interaction of poly[Cys($CH_2COOH$)] with counterions irrespective of their valence or chemical nature.

**Solubilization.** In Figure 5, the state of the solutions, either transparent, turbid, or precipitated, and the polypeptide conformation are shown at $C_p = 1 \times 10^{-4}$ M in Tris buffer ($1 \times 10^{-2}$ M, pH 7.4). In the absence of 0.1 M NaCl (upper zone), solubilization occurred for every surfactant just as is found in the case of PGA.[13] The conformation in the solubilized state was the $\beta$-structure for DAC but random coil for DTAC. The spectra are shown in Figure 1 by dashed curves. On the other hand, CD spectra in the case of DDAC could not be obtained due to strong scattering, suggesting the presence of large aggregates in apparently transparent solutions. The aggregating tendency is stronger for the complex with DDAC than that with DAC or DTAC, which is consistent with that found on the solubilized complexes with PGA.[13]

In the presence of 0.1 M NaCl (lower zone), solubilization occurred only in the case of DAC and the $\beta$-structure was suggested for the solubilized state. However, the CD spectra of the solubilized solution (not shown) were different from other CD curves characteristic of the $\beta$-structure. They exhibited a large red shift (negative band at 233 ± 2 nm, crossover at 225 nm) and suggested the presence of large aggregates and/or environmental effect on peptide chromophore. Solubilization of the DDAC–poly[Cys($CH_2COOH$)] complex was further tried in water (salt-free and unbuffered) but was not successful.

**Irreversible Nature of the Interaction.** To examine whether the induction of the $\beta$-structure occurs reversibly, the following experiments were carried out at $C_p = 1 \times 10^{-4}$ M. Equal volumes of the two solutions of the same polymer concentration but different mixing ratios, $C_D/C_p = 2.0$ (A) and $C_D/C_p = 0$ (B), were mixed to yield a solution (C) ($C_D/C_p = 1$). The CD spectra of solution C were measured 24 h after the mixing and compared with those of a solution (D) of the same composition prepared by the addition of the surfactant stock solution to solution B. The value of $[\theta]_{205}$ of solution C was 6000 ± 2000. This value is significantly larger than the value (~0) found for solution D. This result was interpreted as indicating that free surfactant ions present in solution A were bound to random coils contained in solution B and converted them, to some extent, to the $\beta$-structure. The overall amount of the $\beta$-structure of solution C was thus significantly greater than that of solution D, although these two solutions had the same composition. This result indicates that dissociation of bound surfactant ions is very slow, i.e., that the binding is not reversible practically. The irreversibility with respect

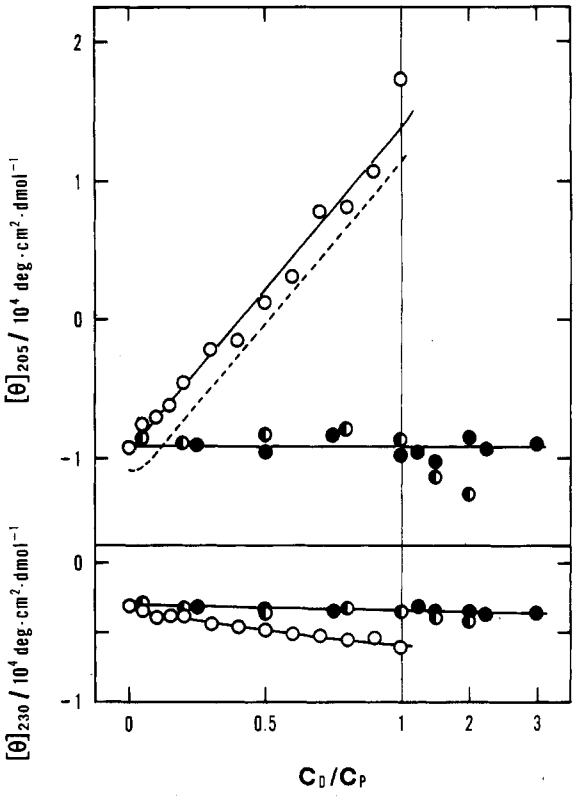


**Figure 6.** Dependence of $[\theta]_{205}$ and $[\theta]_{230}$ on the mixing ratio $C_D/C_p$ in water at $C_p = 1 \times 10^{-3}$ M: (○) DAC, (◐) DDAC, and (●) DTAC. The result at $C_p = 1 \times 10^{-3}$ M in $1 \times 10^{-2}$ M Tris buffer (pH 7.4) is shown for comparison by a dashed curve.

to surfactant binding may be related to the irreversible nature of the stacking of the β-sheets.

**II. Interactions in the Absence of Supporting Electrolytes.** Interaction between opposite charges is enhanced as ionic strength of the media is reduced. As shown in the preceding part, induction of the β-structure does not take place for DDAC or DTAC in $1 \times 10^{-2}$ M Tris buffer. It is pertinent to examine the effects of the three surfactants in unbuffered solutions containing no added salt. The results obtained at $C_p = 1 \times 10^{-3}$ M, shown in Figure 6, indicate the essentially similar effects of the three surfactants. Better resolved CD spectra (not shown) were obtained on these salt-free solutions than given in Figure 1 since the medium is more transparent.

In the absence of supporting electrolytes, interaction of polyions with counterions can be studied by measuring the pH change caused by the addition of salt. In Figure 7, values of pH of the solutions are plotted against the logarithm of surfactant or salt concentration $C$.

A lowering of pH is related to a decrease of the electric surface potential of a polyion. According to an approximate thermodynamic theory,[19] the decrease of pH at constant polyion charges is negligible when $C < C_p$ but linear with log $C$ when $C > C_p$, the slope $[-d(pH)/d(\log C)]$ not exceeding unity. Since counterions from salt and polyion are not identical in the present case, different from the condition assumed in the theory, a deviation from the prediction of the theory is likely to occur. To clarify this point, the addition of KCl was examined. In Figure 7, little change of pH is seen where $C < C_p$ and the slope in the high-concentration range is 0.78. The behavior is consistent with the above theory. Therefore, present data can be approximately interpretated based on the above theory.

Decrease of pH is large in the case of DTAC (the slope is about 1.35). In the case of surfactant ions, hydrophobic interaction among hydrocarbon chains affords a cooperative nature to the binding,[6,20] which is not taken into account in the above theory. Dependence on pH is much larger in the case of DAC and DDAC, the slopes being 3.95 and 2.31, respectively. Marked lowering of pH in the case of these two surfactants suggests that polymer charges are no longer constant but are reduced as a result of their binding. A probable molecular mechanism for the reduction of polymer charges is protonation of carboxylate groups through hydrogen bonds with ammonium head groups. The observed order of lowering of pH for the three surfactants probably reflects any difference in the extent and/or the strength of the assumed hydrogen bonding. However, the order can be also explained by different distances of the closest approach of each surfactant ion to side-chain carboxylates. It should be noted in Figure 7 that there is no discrete difference between DAC and the other two surfactants, although DAC behaves quite differently from the others in the induction of the β-structure.

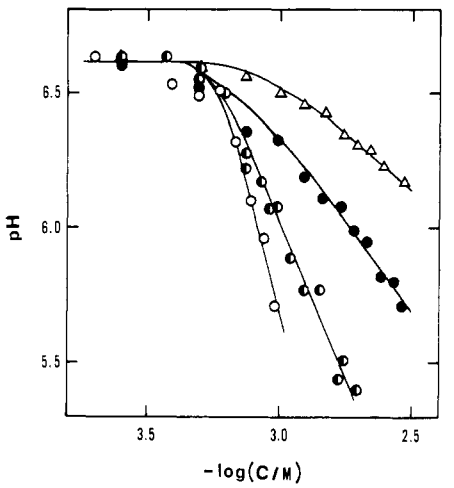


**Figure 7.** Dependence of pH on the logarithm of salt or surfactant concentration $C$ in water at $C_p = 1 \times 10^{-3}$ M: (○) DAC, (◐) DDAC, (●) DTAC, and (△) KCl.

In Figure 6, the result obtained at the same polymer concentration ($C_p = 1 \times 10^{-3}$ M) in $1 \times 10^{-2}$ M Tris buffer (pH 7.4) is also shown in the presence of DAC by a dashed line for the sake of comparison. Although a slight difference exists between the dashed line and the open circles, approximately similar results are obtained. It is clear, therefore, that the decrease of pH shown in Figure 7 is not a cause for the induction of the β-structure but a consequence of specific binding that leads to the induction.

**III. Infrared Absorption Spectra of Poly[Cys(CH$_2$COOH)]-Surfactant Complexes in the Solid State.** To investigate the molecular interaction between surfactants and the polypeptide and also to examine the conformation of the polypeptide in the solid state, infrared absorption spectra were taken on the precipitates produced by the addition of each of the three surfactants. The spectra obtained are shown in Figure 8 and selected data are given in Table I together with relative intensities of carboxylate stretching normalized to the amide A band. The conformation of the polypeptide in the solid state was the β-structure in the complexes with DAC and DDAC, while it was random coil in the case of DTAC, as judged from the amide I band.[21,22] In this way, the conformations in the presence of DAC and DTAC were consistent between the solution and the solid state. On the other hand, the complex with DDAC took the β-structure in the solid state, but it took a random coil in the solution. The prevailing conformation of fully ionized poly[Cys(CH$_2$COOH)] in the solid state is the β-structure even when the counterions are $Na^+$ ions.[23] The distance of the

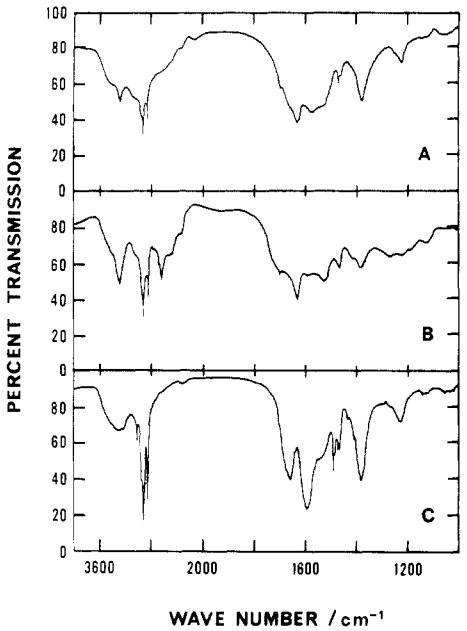


**Figure 8.** Infrared absorption spectra of the complexes of poly[Cys($CH_2$COOH)] with DAC (A), DDAC (B), and DTAC (C).

**Table I**
**Infrared Absorption Bands of Poly[Cys($CH_2$COONa)] in the Complexes with Surfactants**[a]

| | amide I | | | | |
|---|---|---|---|---|---|
| | $\nu(\pi,0)$ | $\nu(0,\pi)$ | amide II | amide A | $\nu_{as}$(COO)[b] |
| Na salt | 1625 | 1699 | 1529 | 3270 | 1584 (2.4) |
| complex with DAC | 1630 | 1699 | 1526 | 3280 | 1575 (1.2 ± 0.2) |
| complex with DDAC | 1630 | 1700 | 1525 | 3280 | 1585 (0.5 ± 0.2) |
| complex with DTAC | 1660 | | 1530 | ~3300[c] | 1587 (3.8)[c] |

[a] Frequency is given in $cm^{-1}$. [b] Antisymmetric stretching band of $COO^-$ group. Relative intensity to amide A band is given in parentheses. [c] Amide A band was not well resolved due to absorption of water.

closest approach of $DTA^+$ to the carboxylate will be large due to the bulky head group, and hence electric repulsive free energy is great enough to destabilize the β-structure. In the DTA salt of a higher side-chain homologue of poly[Cys($CH_2$COOH)], the β-structure is found in the solid state,[24] probably because attractive free energy is greater than in the present polypeptide.

Hydrogen bond formation between the polypeptide and the two surfactants as well as the extent of proton transfer of the hydrogen bond can be examined based on the intensity of the antisymmetric stretching vibration band of the carboxylate group around 1560–1585 $cm^{-1}$.[25,26] The intensity of the band (Table I) was weak for the complex with DDAC or DAC as compared with that of the sodium salt of the polypeptide, which was weaker than that of the complex with DTAC. Hydrogen bond formation and protonation to the carboxylate group were thus indicated in the case of DAC and DDAC. The extent of protonation was greater for DDAC than for DAC in the solid state.

## Discussion

**Induction of the β-Structure in Solution.** From the results on the interaction of bivalent metal cations with poly[Cys($CH_2$COOH)][17] and its side-chain homologue,[18] it is concluded that induction of the β-structure in solution can be achieved only when the charges on the polyions are reduced by a change in the electronic state, such as by protonation in the case of hydrogen counterions or by coordination of transition-metal counterions.

On the basis of the IR results on the solid state as well as on the pH change in solution (Figure 7), it is strongly suggested that a hydrogen bond is formed between the carboxylate group and a $DA^+$ or a $DDA^+$ cation and that proton transfer occurs between them, more or less, even in solution. On the other hand, $DTA^+$ cations cannot form a hydrogen bond and are bound to the polyions electrostatically, although cooperative binding occurs due to hydrophobic interaction among their hydrocarbon chains. According to the working hypothesis mentioned above, the induction of the β-structure of poly[Cys($CH_2$COOH)] in solution can occur for DAC and DDAC if the extent of protonation is large enough. Present results suggest that proton transfer in solution occurs more extensively for DAC than for DDAC. On the other hand, enhancement of the binding due to the cooperative nature turns out to be insufficient to induce the β-structure.

Comparison of the inducing power of DAC with that of a transition-metal cation[17] could be done at $C_p = 1 \times 10^{-3}$ M in water (no added salt, unbuffered) with a reservation that the chain lengths of the samples employed are 360 in the present study but 75 in the previous study.[17] The mixing (molar) ratios that gave $1 \times 10^4$ for $[\theta]_{200}$ were about 0.27, 0.34, 0.37, 0.50, and 0.6 for $Cu^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Ni^{2+}$, and $Co^{2+}$, respectively.[17] In the case of DAC, $[\theta]_{205}$ varies linearly with $C_D/C_p$ as shown in Figure 6. The ratio $[\theta]_{200}/[\theta]_{205}$ for the β-structure of poly[Cys($CH_2$COOH)] has been established to be about 2. Therefore, the value of $C_D/C_p$ corresponding to $[\theta]_{205} = 5 \times 10^3$ (where $[\theta]_{200}$ is expected to be $1 \times 10^4$) is about 0.6 in Figure 6. Consequently, the induction power of DAC is comparable with that of $Co^{2+}$, or somewhat weaker if the difference of chain lengths is taken into account. This estimate is further supported from the dependence of pH. Decrease of pH occurred in the concentration range of $Ni^{2+}$ or $Co^{2+}$ ions lower than $5 \times 10^{-4}$ M,[17] while pH remains nearly constant in the same concentration range of DAC. Similar comparison can be carried out between DAC and $Ag^+$ ions.[27] The inducing power of DAC is somewhat weaker than that of $Ag^+$. These results are quite reasonable, since $DA^+$ ion are univalent and metal cations form covalent bonds while the covalent nature of $DA^+$ binding is weak unless proton transfer through the hydrogen bond occurs completely. In aqueous solutions, the polar environment favors a polarized stated[28,29] and hence protonation of carboxylate groups is greatly reduced in the solution as compared with the solid state. The extent of proton transfer of DDAC is larger in the solid state but smaller in solution than that of DAC. Since the distance of closest approach is longer for $DDA^+$ than for $DA^+$, the $DDA^+$–carboxylate complex is more susceptible to the perturbation of water.

Since the DDAC–poly[Cys($CH_2$COOH)] complex formed the β-structure in the solid state, it was hoped that the β-structure could be observed in a solubilized solution. However, solubilization occurred only in Tris buffer ($1 \times 10^{-2}$ M, pH 7.4) where CD spectra could not be obtained due to strong scattering.

**Characteristic Features of the β-Structure and the α-Helix As Revealed in Their Interactions with Surfactants.** When the present study is compared with the previous one,[13] a significant difference between the α-helix and the β-structure shows up through their interactions with surfactant counterions, particularly with respect to the specificity about their head groups, the effect

of ionic strength, and the effect of polypeptide concentration. Interactions participating in the secondary structures of polypeptides, either repulsive or attractive, are stronger in the case of the $\beta$-structure as compared with the $\alpha$-helix. From the potentiometric titration data, for example, electric repulsive free energy is much larger for the $\beta$-structure than for the $\alpha$-helix when compared at the same degree of ionization.[30-35] Also, nonelectric stabilizing contributions evaluated from the area of titration curves are much larger in the case of the $\beta$-structure than the $\alpha$-helix.[30-35] Induction of the $\beta$-structure hence requires a net reduction of polymer charge density to a greater extent than that of the $\alpha$-helix. Molecular interactions such as coordination, hydrogen bonding, and the resulting extent of proton transfer can be sensitively monitored in dilute solutions by using a secondary structure of the polypeptide as a probe. Depending on the range of the strength of interaction in question, either $\alpha$- or $\beta$-induction can be used. Interaction with bivalent metal cations provides essentially identical information for the induction of either the $\alpha$-helix (PGA)[36] or the $\beta$-structure;[17] transition-metal cations were effective, while alkaline earth metal cations were ineffective. This is because the difference between the two groups is so large that different stabilities of the two secondary structures do not show up. On the contrary, our understanding of the interaction with the three cationic surfactants is deepened when the results for poly[Cys($CH_2$COOH)] and PGA are combined. The interaction with PGA discriminates DTAC from DAC and DDAC insofar as the induction of the $\alpha$-helix is concerned, while that with poly[Cys($CH_2$COOH)] discriminates DAC from DDAC and DTAC more clearly. Generally, the induction of the $\alpha$-helix works in monitoring a weak interaction while a strong interaction is well monitored by the induction of the $\beta$-structure.

**Effect of Added Salt.** In the previous study,[13] the induction of the $\alpha$-helix was largely suppressed in the presence of 0.1 M NaCl, while the induction of the $\beta$-structure was scarcely affected in the same solvent, as shown in the present study. The different effects of ionic strength imply different binding free energies. Since the overall binding reaction involves a conformational change of the polypeptide, different binding constants for the same interaction pair (carboxylate–counterion) are expected for different conformational changes. The free energy gain on counterion binding is much greater in the case of the $\beta$-structure than the $\alpha$-helix, since both attractive and repulsive interactions are greater for the former than the latter. This is equivalent to a stronger binding constant for the $\beta$-structure than for the $\alpha$-helix. The different effects of ionic strength are thus interpreted as a result of the different overall binding constants between these two secondary structures. Different intrinsic dissociation constants of the carboxyl groups of these two polypeptides, $pK_0 = 4.5$ for PGA[30-32] and 3.2 for poly[Cys($CH_2$COOH)],[34] would result in a behavior opposite to the observed one, and hence it is not a cause for the observed different binding constants.

**Registry No.** DAC, 929-73-7; DDAC, 2016-48-0; DTAC, 112-00-5; poly[Cys($CH_2$COONa)] (homopolymer), 33989-65-0; poly[Cys($CH_2$COONa)] (SRU), 84809-36-9; poly[Cys($CH_2$COOH)] (homopolymer), 29433-95-2; poly[Cys($CH_2$COOH)] (SRU), 31851-29-3.

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