Conformation of Poly(L-glutamic acid) in Solutions of a Cationic Surfactant with an ω-Hydroxyl Group

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The conformation of poly(L-glutamic acid) was examined based on the circular dichroism spectra in solutions of ω-hydroxyalkylammonium bromides with the dodecyl or undecyl chain and the methyl-, dimethyl- or trimethylammonium moiety, as well as in normal surfactant solutions of dodecylammonium bromides with a corresponding cationic group used as a reference. The conformational change from a random coil to an α-helix was observed, depending on the chain length and head group of the surfactant. A surfactant with a longer chain length induced the α-helix at a lower concentration. A surfactant with more methyl groups has less ability for inducing an ordered conformation, presumably due to a steric hindrance, though it is more hydrophobic. No ordered conformation of poly(L-glutamic acid) was observed in surfactants with the trimethylammonium cationic group.

Since poly(L-lysine) was found to induce the β-form in a solution of sodium dodecyl sulfate,[1] many studies have been devoted to conformational changes of polypeptides induced in surfactant solutions.[2—21] These extensive studies have revealed that dissociative polypeptides interact cooperatively with surfactant ions of opposite charge and induce a conformational change in the polypeptide at low concentrations of the surfactant, far below the critical micelle concentration (cmc).[22—24] The cooperative binding of surfactant ions arises from a 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. We recently reported that the conformational change of a polypeptide strongly couples with cooperative binding of a surfactant.[4,7]

Poly(L-glutamic acid) (P(Glu)) also cooperatively interacts with dodecylammonium ions and induces an α-helix.[23] Maeda et al. also investigated the conformation of P(Glu) in solutions of dodecyltrimethylammonium chloride (DTAC, $C_{12}H_{25}(CH_3)_3NCl$)), dodecyldimethylammonium chloride (DDAC, $C_{12}H_{25}(CH_3)_2NHCl$) and dodecylammonium chloride (DAC, $C_{12}H_{25}NH_3Cl$) at the surfactant concentrations unto the precipitation region of P(Glu)-surfactant complex; they found an α-helix as well as an aggregation of α-helices of P(Glu).[8] Though a much stronger and highly cooperative interaction is expected, hexadecyltrimethylammonium bromide induces no ordered conformation of P(Glu).[25]

In the present study we examined the conformation of P(Glu) in solutions of more hydrophilic cationic amphiphiles that have a hydroxyl group at the ω-position. They are (12-hydroxydodecyl)dimethylammonium chloride (HDDAC, $HOC_{12}H_{24}(CH_3)_2NHCl$), (12-hydroxydodecyl)methylammonium chloride (HDMAC, $HOC_{12}H_{24}(CH_3)NH_2Cl$), (11-hydroxyundecyl)trimethylammonium chloride (HUTAC, $HOC_{11}H_{22}(CH_3)_3NCl$), (11-hydroxyundecyl)dimethylammonium chloride (HUDAC, $HOC_{11}H_{22}(CH_3)_2NHCl$), and (11-hydroxyundecyl)methylammonium chloride (HUMAC, $HOC_{11}H_{22}(CH_3)NH_2Cl$). These surfactants show no micellization up to 0.1 mol dm^{-3} concentration. We also reexamined the conformation of P(Glu) in solutions of DTAC, DDAC, and dodecylmethylammonium chloride (DMAC, $C_{12}H_{25}(CH_3)NH_2Cl$).

Experimental

Materials. Sodium salt of poly(L-glutamic acid) (P(Glu)) was purchased from Peptide Laboratories, Osaka, Japan. Dodecyltrimethylammonium bromide (GR) was purchased from Tokyo Kasei and recrystallized three times from acetone. 12-bromo-1-dodecanol and 11-bromo-1-undecanol were purchased from Aldrich, and used for syntheses of the corresponding ω-hydroxyalkylamines without further purification. 1-bromododecane was purchased from Tokyo Kasei and used for syntheses of dodecyldimethylammonium bromide and dodecylmethylammonium bromide. Hydrochlorides of trimethylamine (GR), dimethylamine (GR), and methylamine (GR) were obtained commercially and used as received.

The surfactant bromides were prepared by reacting ω-bromo alcohol with the corresponding alkylamine,[26] and were ion-exchanged to the chlorides. The mass spectra confirmed the corresponding amines.

Measurements. P(Glu) was dissolved in a 0.1 mol dm^{-3} NaOH solution and was thoroughly dialyzed against deionized water. The equivalent concentration was determined by colloid titration. The bromides of the surfactant were ion-exchanged into their chlorides, because the bromide interferes with a measurement of the circular dichroism (CD) spectra of P(Glu). CD spectra were recorded using a JASCO J-20A polarimeter. The concentration of P(Glu) was kept constant at 0.28 mmol dm^{-3}.

Results and Discussion

Conformation of P(Glu) in the ω-Hydroxyalkylammonium Solutions. The conformation of P(Glu) was determined by the CD spectrum in the presence of excess cationic surfactants at neutral pH. Table 1 summarizes the results. The dodecylammonium salts were reexamined for the reference. All of the meth-

Table 1. Conformation of Poly(L-glutamic acid) in Solutions of Cationic Surfactant at a Neutral pH

Tail \ Head	Trimethylammonium chloride	Dimethylammonium chloride	Methylammonium chloride
11-Hydroxyundecyl-	Coil	Coil	Helix
12-Hydroxydodecyl-	Coil	Helix	Helix
Dodecyl-	Coil (Helix)[a)]	Helix	Helix

a) Ref. 8.

ylammonium salts induce the α-helix of P(Glu), while all of the trimethylammonium salts induce no ordered conformation. The dimethylammonium salts induce an α-helix by HDDAC and DDAC, but not by HUDAC.

The CD spectrum ascribed to the ordered conformation was not found below the DTAC concentration where precipitation of a complex of P(Glu) with DTAC was found; further, Maeda et al. found an α-helix in a DTAC solution.[8)] We examined hexadecyltrimethylammonium chloride with a longer hydrophobic tail and confirmed that it induced no orderd conformation of P(Glu), as found by Mattice et al.[25)] Another example is poly[*S*-(2-carboxyethyl)-L-cysteine]. It formed no ordered conformation in a trimethylammonium salt DTAC solution, but induced a β-sheet in a dodecylammonium chloride solution.[27)] We therefore conclude that the trimethylammonium salts of a surfactant induce no ordered conformation, presumably due to a steric hindrance.

HDDAC, with a longer hydrophobic tail, induces an α-helix of P(Glu), while HUDAC induces no ordered conformation. Satake and Yang found an α-helix of poly(L-ornithine) in solutions of alkyl sulfates with the different chain lengths, $CH_3(CH_2)_nOSO_3Na$ with n=7, 9, 11, 13, and 15; however, the magnitude of the CD extremes of an α-helix decreases with decreasing the chain length of the surfactant.[19)] A surfactant with a shorter chain length induced a no more ordered conformation of the polypeptide. Taking into account their results, we infer no induction of the ordered conformation of P(Glu) by ω-hydroxyalkyldimethylammonium salts with a shorter hydrophobic chain than that of the dodecyl group. Decylammonium chloride has a shorter chain length and induces an α-helix of P(Glu),[8,23)] since it has no hydroxyl group at the ω-position and is more hydrophobic than HUDAC.

Dependence of the Conformation of P(Glu) on the Surfactant Concentration. Figure 1 shows the CD spectra of P(Glu) solutions in the presence of different amounts of HDDAC. Spectrum a in the absence of HDDAC represents the state of a charged random coil. As the HDDAC concentration increases, an α-helix is induced, as shown by the change in the CD spectra (curves b, c, d, and e). Spectrum e, having a double minima, is characteristic of an α-helical conformation. The fact that the isoelipticity point is observed at 205 nm indicates a single conformational change from a random coil to an α-helix.

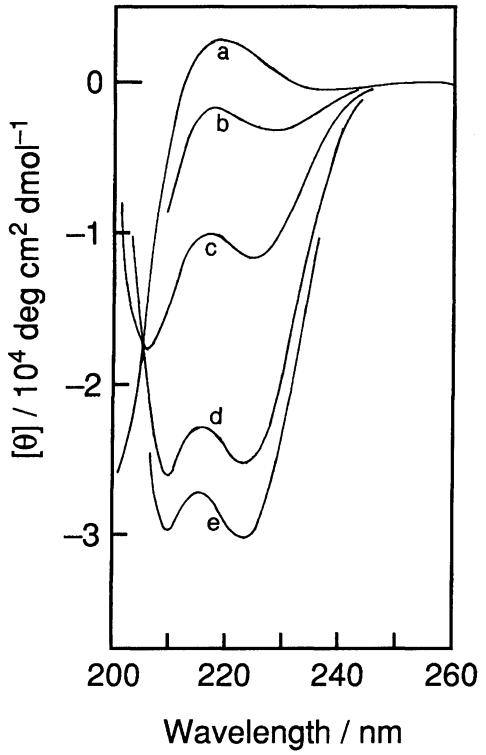

Fig. 1. CD spectra of P(Glu) in the solutions containing various amounts of HDDAC at a neutral pH and 25 °C. No added salt. The glutamyl residue concentration (C_P): 0.28 mmol dm^{-3}. The molar ratio of added HDDAC to L-glutamyl residues (C_S/C_P): a 0, b 8.75, c 11.7, d 14.6, e 20.4.

The CD spectra of P(Glu) in HDMAC and HUMAC solutions also indicate a conformational change from a random coil (a) to an α-helix (e) as the surfactant concentration increases (Figs. 2 and 3). These spectra also show an isoelipticity point at 205 nm, again indicating a single conformational change of P(Glu) from a random coil to an α-helix. A further increase in the surfactant concentration induces a spectrum having a single minimum at 223 nm (curve f in Fig. 3), or a spectrum having a minimum at 225 nm and a shoulder at 210 nm (curve f in Fig. 2). After exhibiting these distorted spectra, the solution sometimes became turbid with passing time, or a further increase of the surfactant. These spectra

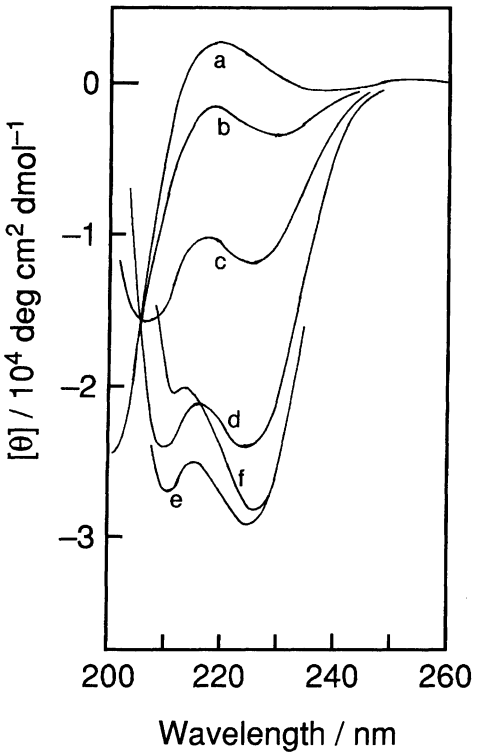

Fig. 2. CD spectra of P(Glu) in the solutions containing various amounts of HDMAC at a neutral pH and 25 °C. No added salt. C_P: 0.28 mmol dm^{-3}. C_S/C_P: a 0, b 4.05, c 4.86, d 6.49, e 12.2, f 24.3.

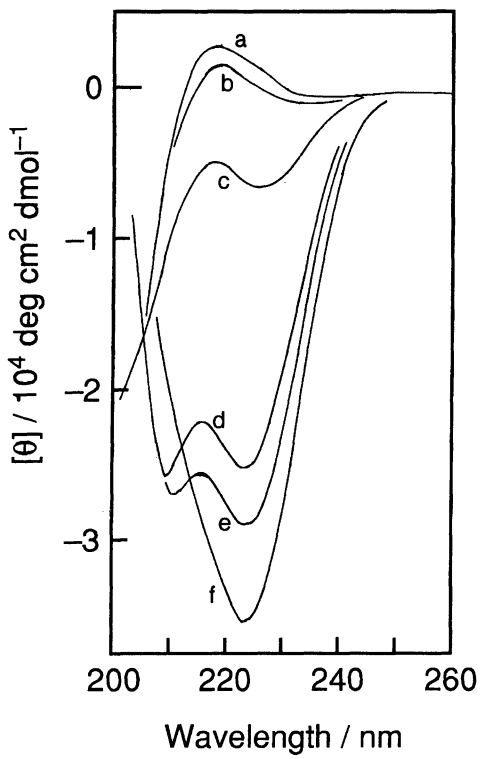

Fig. 3. CD spectra of P(Glu) in the solutions containing various amounts of HUMAC at a neutral pH and 25 °C. No added salt. C_P: 0.28 mmol dm^{-3}. C_S/C_P: a 0, b 21.0, c 27.3, d 42.0, e 52.5, f 78.8.

indicate a truncation of the double extremes at shorter wavelengths and a shift of the extremes at 222 nm to the longer wavelength side. These spectra resemble the calculated CD spectra of aggregates of helical P(Glu) when they are approximated by spheres having a radius of 0.03—0.1 μm.[28] A similar spectrum of P(Glu) was also observed at a higher mixing ratio of surfactant to glutamyl residues in DAC and DDAC solutions, and was ascribed to aggregates of α-helices of P(Glu).[8,25] Maeda and coworkers also found a similar distorted CD spectra in solutions of various bivalent metal cations.[29] From the IR spectra of the precipitates, which showed a distorted CD spectrum, and a difficulty to dissolute the β-precipitate of P(Glu),[30] they concluded that the conformation of P(Glu) in soluble aggregates is the α-helix. Therefore, both spectra f in Figs. 2 and 3 are ascribed not to the β-sheet, but to aggregates of the α-helices of P(Glu). A cationic polypeptide, poly(L-ornithine), also induced a similar spectrum in sodium alkanesulfonate solutions after the spectrum of a typical α-helix as the surfactant concentration was increased.[24] Thus, P(Glu) induces a single conformational change from a random coil to an α-helix in a solution of these three ω-hydroxyl surfactants.

The dependence of the conformational change of P(Glu) on the surfactant concentration is compared with the result by the corresponding normal surfactant in Fig. 4. The extent of α-helices is measured based on the residue ellipticity at 222 nm, $[\theta]_{222}$. It increases with decreasing $[\theta]_{222}$. In the case of DDAC (b in Fig. 4), the onset of a conformational change in the α-helix occurs immediately after the addition of a surfactant; in case of HDDAC (a in Fig. 4), however, P(Glu) stays on the random coil until an excess amount of the surfactant is added. In the case of HDMAC, the onset of a conformational change in the α-helix occurs at a molar ratio of the surfactant to the peptide residue (C_S/C_P) of 4, while in the case of DMAC it occurs when a very small amount of DMAC is added. These observations indicate the weaker interaction of the ω-hydroxyalkylammoniums with P(Glu) than that of the corresponding normal surfactants. Since the ω-hydroxyalkylammonium equals to the corresponding normal surfactant regarding the electrostatic contribution, the weaker interaction of the former arises due to their hydrophilic property. HDDAC and HDMAC are more stable in aqueous bulk than are DDAC and DMAC, respectively. However, a more hydrophilic surfactant with two ionic groups at the α- and ω-positions strongly interact with polypeptides of opposite charge due to their strong ionic interaction. For instance, poly(L-lysine) induces an α-helix at a surfactant/lysine residue ratio of about 0.6 in disodium 1,12 dodecanediyl disulfate solution, but no ordered conformation in disodium 1,10-decanediyl disulfate solution, even at a ratio at which the complex of P(Glu) precipitates.[7] This observation shows

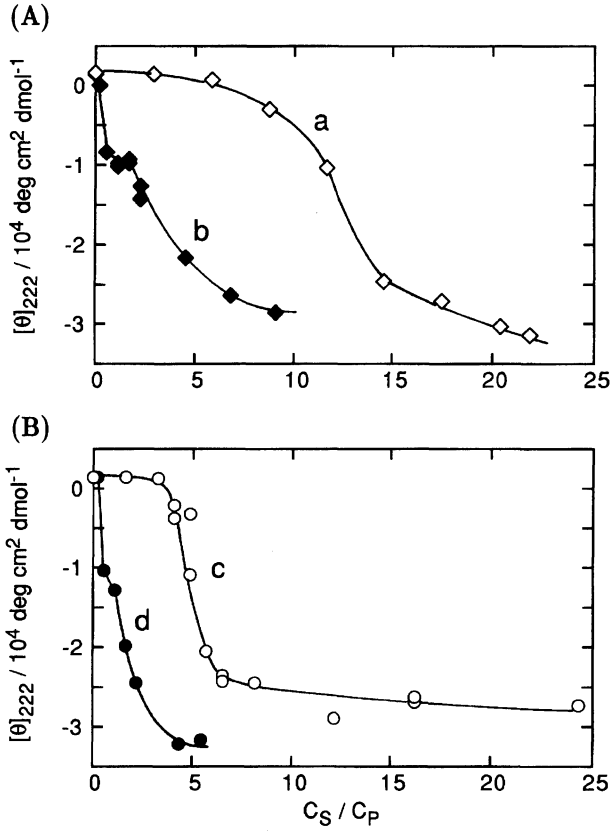

Fig. 4. Surfactant concentration dependence of the mean residue elipticity at 222 nm for P(Glu) at a neutral pH and 25 °C in various surfactant solutions. C_P: 0.28 mmol dm^{-3}. Surfactant: a HDDAC, b DDAC, c HDMAC, d DMAC.

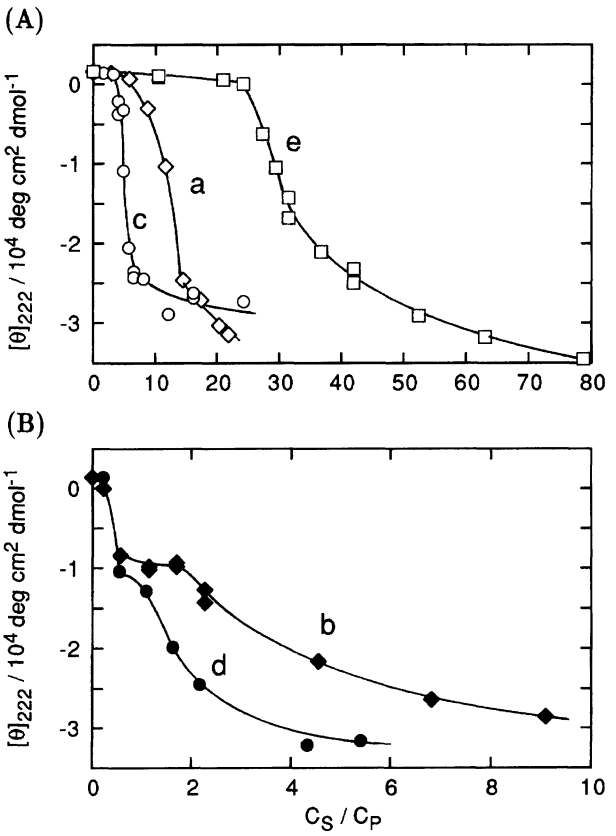

Fig. 5. Surfactant concentration dependence of the mean residue elipticity at 222 nm for P(Glu) at a neutral pH and 25 °C in various surfactant solutions. C_P: 0.28 mmol dm^{-3}. Surfactant: a HDDAC, b DDAC, c HDMAC, d DMAC, e HUMAC.

that charge neutralization by surfactant binding is not enough to induce an ordered conformation of a polypeptide. The induction of an ordered conformation of a polypeptide also requires hydrophobic surroundings by the surfactant.[4,7] The ω-hydroxyalkylammonium ions interact weakly with the polyelectrolytes of opposite charge[26] and, furthermore, have a weak ability to form a hydrophobic domain on P(Glu).

Figure 5 shows a comparison of the conformational change due to surfactants with a different ionic head group. A sharp change in the α-helix occurs at $C_S/C_P=5$ in an HDMAC solution, while a gradual change occurs at a molar ratio of 10 to 18 in an HDDAC solution. Since HDDAC has one more methyl group and is more hydrophobic than HDMAC, we may expect that it is more effective to form a hydrophobic environment on P(Glu), and also for the induction of an α-helix. In fact, HDDAC is less effective for the induction of the α-helix of P(Glu). This is due to a steric hindrance produced by a larger head size of the dimethylammonium group. Trimethylalkylammonium, even with a longer alkyl chain, for instance hexadecyltrimethylammonium chloride, induces no ordered conformation (as described above). A similar discussion may be valid for a comparison between normal surfactants, DDAC and DMAC, as shown in Fig. 5B.

Since the x-axis is extended in Fig. 5B, we can observe the onset of a conformational change at a molar ratio of 0.4 and a clear stepwise change along with increasing the surfactant contents in both DDAC and DMAC solutions. Mattice et al. also observed a similar stepwise transition in a dodecylammonium chloride solution.[25]

A comparison of curves c and e in Fig. 5A indicates that the effect of the chain length of ω-hydoxyalkylammonium on the induction of the α-helix is quite large. One reason for this is the weaker binding of HUMAC to P(Glu). According to our results concerning a highly cooperative binding system of a normal surfactant ion by a polyion of opposite charge, the onset of binding occurred at a 3.5-times lower concentration of the surfactant while increasing one methylene group in the surfactant tail.[31,32] In the present case, the onset of a conformational change to the α-helix occurs at a 6-times lower concentration in HDMAC than in HUMAC. Here, we must take into account a deviation between the surfactant binding and the conformational change. The degree of the α-helix of poly(L-ornithine) increased linearly with increasing the degree of the binding of sodium dodecanesulfonate, which means that a con-

formational change starts with the onset of surfactant binding. However, in sodium nonanesulfonate, having a shorter tail, onset of the conformational change occurred at a 0.5 binding degree of the surfactant.[24] This was ascribed to less cooperative binding and the smaller ability to form a hydrophobic surrounding of the latter surfactant.[4] Therefore, the deviation for a conformational change to an α-helix between HDMAC and HUMAC is partly due to the formation of a less hydrophobic surrounding of HUMAC. A theoretical treatment indicated that a more cooperative binding of a surfactant with a long chain is more effective for the α-helix transition of poly(L-ornithine).[19]

Finally, a rough estimation of the free energy change in the conformational change of P(Glu) was attempted based on the following simple mechanism. For each site, the following overall reaction is taken into account:

$$c + S^+ \rightleftharpoons h, \quad (1)$$

where c and h stand for the random coil site and the helix site in P(Glu), respectively, and S^+ for a surfactant cation. Since the present surfactants with an ω-hydroxyl group form no micelle, and regular surfactants were used at a concentration far below the cmc, micellization was excluded. At the midpoint of the conformational change of P(Glu), the number of helix sites equals that of random coil sites. The following relationship can be derived:

$$(h)/(c) = K[S]_e = 1. \quad (2)$$

where the parentheses stand for the concentration of each site, $[S]_e$ is the equilibrium concentration of the surfactant and K is the apparent equilibrium constant of Reaction 1 at the midpoint. The free energy change can be approximately estimated based on the equilibrium concentration of the surfactant at the midpoint of the conformational change. Though we are not in a situation to measure $[S]_e$, we can read the added surfactant concentration at the midpoint of the conformational change as being 9.0 mmol dm^{-3} for HUMAC, 1.4 mmol dm^{-3} for HDMAC, 3.4 mmol dm^{-3} for HDDAC, 0.84 mmol dm^{-3} for DDAC and 0.42 mmol dm^{-3} for DMAC. When the concentration of the surfactant is much larger than that of P(Glu), $[S]_e$ may be set as the added concentration of the surfactant at the midpoint of the conformational change; a rough estimation of K is therefore possible. From the ratio of K, we estimate that the free energy change for Reaction 1 decreases 2.2 kJ mol^{-1} for a change in the head group from HDDAC to HDMAC and 4.6 kJ mol^{-1} for a change in the chain length from HUMAC to HDMAC. Since the surfactants are not in excess of P(Glu) in the DDAC and DMAC systems, we assume that half of the glutamyl residues are occupied by the cationic surfactant at the midpoint of the conformational change, where $[S]_e$ is estimated to be 0.70 mmol dm^{-3} for DDAC and 0.28 mmol dm^{-3} for DMAC; an estimate of the free energy decrease is 2.3 kJ mol^{-1} for a change of the head group from DDAC to DMAC. An increase of one methyl group in the head of alkylammonium surfactant leads to a free energy loss of about 2 kJ mol^{-1}, while the increase of one methylene group in the surfactant chain leads to a 4.6 kJ mol^{-1} free energy gain. (Note that the free energy change is given for the overall reaction, which includes that for surfactant binding as well as for the conformational change at the midpoint of the conformational change of P(Glu).) When we take into account that the increase of one methylene group in sodium alkanesulfonate surfactants leads to a 3.7 kJ mol^{-1} free energy gain for the binding of the surfactant by poly(L-ornithine),[24] about 1 kJ mol^{-1} for the latter free energy gain is expected to be due to the conformational change of P(Glu).

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