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ORIGINAL CONTRIBUTION

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Conformation of poly(L-glutamic acid) in aqueous solutions of linear amphiphiles with a cationic group at both ends

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Abstract The conformations of poly(L-glutamic acid) [P(Glu)] in solutions of the bipolar amphiphile 1,20-icosanediylbis(alkylammonium chloride) [C20(RA)$_2$], where RA includes trimethylammonium (TMA), dimethylammonium (DMA), or methylammonium (MA), were investigated with measurements of the circular dichroism spectra at 10–35 °C. All C20(RA)$_2$ induced an α-helix of P(Glu) in the aqueous solutions. The residue molar ellipticity at 222 nm showed a similar dependence on the amphiphile concentration (C_s) below 0.5 of the ratio of $2C_s$ to the residue concentration (C_p) of P(Glu), but it separated into three directions at $2C_s/C_p > 0.5$. C20(MA)$_2$ induced an α-helix of P(Glu) at $2C_s/C_p < 0.5$ followed by a helix aggregate at $2C_s/C_p > 0.5$. C20(DMA)$_2$ and C20(TMA)$_2$ also induced an α-helix, but a helix aggregate. C20(TMA)$_2$ indicated a strong temperature dependence and did not induce a complete α-helix at 35 °C.

Keywords Polypeptide conformation · Poly(L-glutamic acid) · Bolaform surfactant · Circular dichroism spectra

Introduction

The conformation of polypeptides under different conditions provides fundamental information on protein conformation. Since the discovery of the conformational change of polypeptides in denaturant surfactants, which induce the ordered conformation of ionic polypeptides, many theoretical and experimental studies have been carried out [1–12]. The ordered conformation of ionic polypeptides is induced in a variety of surfactant solutions, and depends on surfactant structure, hydrophile–lipophile balance, hydrophobic chain length, added salts, and temperature [3, 4, 6, 13, 14]. We have studied the conformation of ionic polypeptides in aqueous solutions of various surfactants, coupled with the cooperative binding of the surfactants by the polypeptides, and proposed the significant roles of the formation of a hydrophobic environment around the polypeptide backbone and ionic neutralization [15–18]. In past studies, we did not find any ordered conformation of poly(L-glutamic acid) [P(Glu)] with alkyltrimethylammonium ions [19, 20], but Maeda et al. [10] found a helix conformation in cationic surfactant solutions. In this study, we revisit the circular dichroism (CD) spectra of P(Glu) in cationic amphiphiles. We used a special group of amphiphiles with a cationic group at both ends, because a strong interaction with P(Glu) was expected. The structure of the hydrophilic head was found to influence the conformational change of P(Glu).

Experimental

Materials

P(Glu) ($M_r > 8{,}000$, Peptide Institute, Osaka) in 2 M NaCl solution was repeatedly dialyzed against freshly distilled water until no chloride was detected. The concentrations of the ionic groups were determined by colloid titration. The compounds 11-bromoundecanoic acid (TCI, Tokyo), sodium hydride (Aldrich), trimethyl ammonium chloride (Wako, Osaka), dimethylamine hydrochloride

(Wako, Osaka), and methylamine hydrochloride (Wako, Osaka) were used to synthesize bipolar amphiphiles, without further purification.

Synthesis of bipolar amphiphiles

The bipolar amphiphiles 1,20-icosanediylbis(alkylammonium chloride) [C20(RA)$_2$], where RA includes trimethylammonium (TMA), dimethylammonium (DMA), or methylammonium (MA), were synthesized from 1,20-dibromoicosane, which was synthesized by the Kolbe electrolysis of 11-bromoundecanoic acid. Kolbe electrolysis was carried out in NaOH–methanol solutions at 0.2 A for 10 h at temperatures below 20 °C in a cooling bath. The crude product was recrystallized from ethanol, and the IR spectrum of the purified material showed the disappearance of the –CO band. The mixture of the product and the corresponding alkylamine was refluxed at 70 °C for 1 h for TMA and DMA salts, and at 80 °C for 4 h for the MA salt. A solid appeared at −18 °C and was repeatedly recrystallized from acetone including hydrochloric acid. The yield was 60% for C20(TMA)$_2$, 40% for C20(DMA)$_2$, and 50% for C20(MA)$_2$. The products were confirmed by ^{1}H NMR spectroscopy.

Measurements

CD spectra of P(Glu) were measured in aqueous solutions of C20(RA)$_2$ at various temperatures, using a JASCO J-500 CD spectrometer with a thermoregulatory attachment. Slow time dependence of the CD intensity has often been observed [17] and the CD spectrum was recorded after at least 1 h of mixing at a given temperature. The IR and ^{1}H NMR spectra were recorded using JASCO FT/IR 500 and JEOL SCM 400 spectrometers, respectively. The solution pH was controlled at 8 by sodium hydroxide. The concentration of P(Glu) was kept constant at 0.2 mM residue.

Results and discussion

CD spectra

The CD spectrum of an L-type polypeptide around 220 nm is a function of its conformation. The random-coil conformation gives a CD spectrum with a small positive maximum at 216 nm and a strong negative minimum at 197 nm. The ordered conformation of an α-helix gives a CD spectrum with a strong negative double minimum at 207 and 222 nm, while a β-sheet gives one with a single minimum at 217 nm. Both ordered conformations indicate CD spectra with a strong positive maximum below 200 nm. We can distinguish the conformation of polypeptides under various conditions from the CD spectrum.

The CD spectra of 0.2 mM P(Glu) in C20(MA)$_2$ solution are shown in Fig. 1 as a function of the amphiphile concentration at 10 °C. The concentration of P(Glu) (C_p) is given on a residue molar basis and the amphiphile concentration is given as an equivalent ratio ($2C_s/C_p$) to P(Glu). Spectrum 1 in the absence of C20(MA)$_2$ represents the random-coil conformation of charged P(Glu). In the presence of C20(MA)$_2$, the

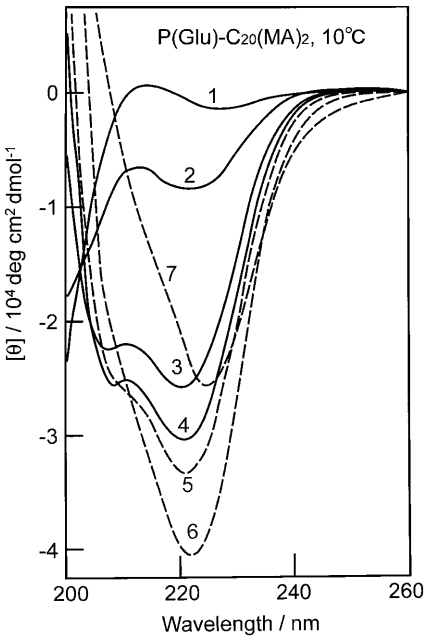

Fig. 1 Circular dichroism (*CD*) spectra of poly(L-glutamic acid) [P(Glu)] in C20(MA)$_2$ solutions at various $2C_s/C_p$ ratios at 10 °C. $2C_s/C_p$: *1* 0.00; *2* 0.20,;*3* 0.53; *4* 0.87; *5* 1.11; *6* 1.76; *7* 2.50

spectrum decreased in the range 204–240 nm, and spectrum 4, with double minima at 207 and 222 nm, was obtained at $2C_s/C_p = 0.87$. The spectrum is slightly deformed compared to the typical CD spectrum of the α-helix conformation of P(Glu) [21]. A further increase in C20(MA)$_2$ enhanced the CD intensity at 222 nm and decreased the intensity at 208 nm (spectra 5–7 in Fig. 1). At $2C_s/C_p > 1.5$, the CD intensity at 208 nm decreased quickly compared to the decrease at 202 nm, and a single minimum spectrum appeared. A similar spectral change was observed for poly(L-ornithine) in aqueous solutions of sodium undecanesulfonate, for which the spectrum with a single minimum was ascribed to helical aggregates [17]. Spectra 1–3 have an isoelliptic point at 203 nm, indicating a conformational change between the two typical states: a random coil and the α-helix. The fact that no isoelliptic point is observed in spectra 4–7 indicates a complex change, involving at least three states. The process of aggregation of the helical P(Glu) may be responsible for the spectral change.

The CD spectra of P(Glu) in C20(MA)$_2$ solutions at 25 and 35 °C are shown in Figs. 2 and 3, respectively. Both figures also show an isoelliptic point at 203 nm at a low $2C_s/C_p$ ratio and the disappearance of the minimum at shorter wavelengths at a high $2C_s/C_p$ ratio, suggesting the conformational change of P(Glu) from a random coil to an α-helix, and then to a helical aggregate. The CD intensity tended to decrease slightly as the solution temperature increased.

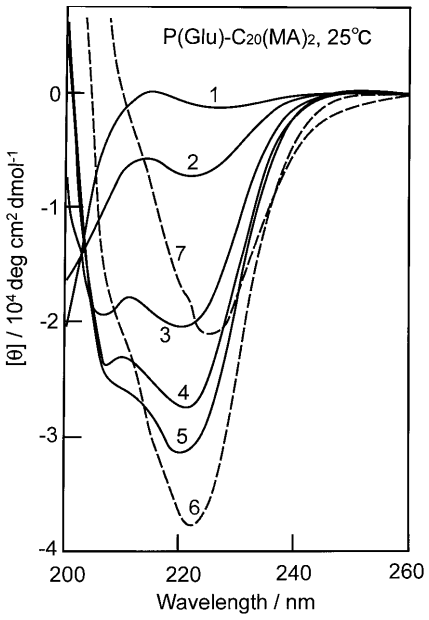

Fig. 2 CD spectra of P(Glu) in C20(MA)$_2$ solutions at various $2C_s/C_p$ ratios at 25 °C. $2C_s/C_p$: *1* 0.00; *2* 0.20; *3* 0.53; *4* 0.87; 5 1.11; *6* 1.76; *7* 2.50

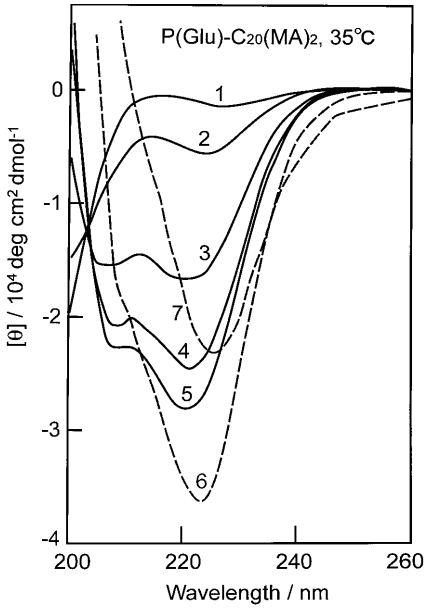

Fig. 3 CD spectra of P(Glu) in C20(MA)$_2$ solutions at various $2C_s/C_p$ ratios at 35 °C. $2C_s/C_p$: *1* 0.00; *2* 0.20; *3* 0.53; *4* 0.87; *5* 1.11; *6* 1.76; *7* 2.50

The CD spectra of P(Glu) in C20(DMA)$_2$ solutions at 10 and 35 °C are shown in Figs. 4 and 5, respectively. They also include spectra with double minima, indicating a conformational change from a random coil to an α-helix as the amphiphile concentration increases. These spectra also show an isoelliptic point around 202 nm at

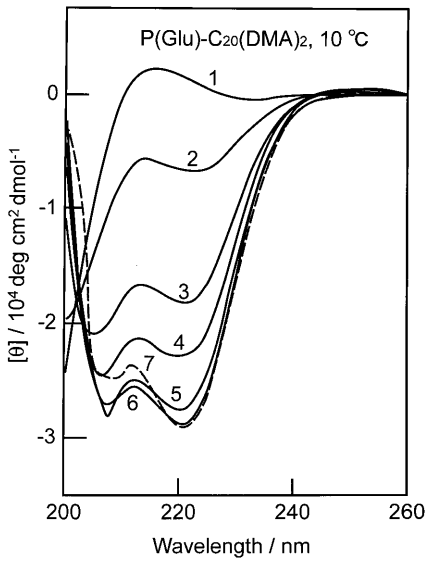

Fig. 4 CD spectra of P(Glu) in C20(DMA)$_2$ solutions at various $2C_s/C_p$ ratios at 10 °C. $2C_s/C_p$: *1* 0.00; *2* 0.20; *3* 0.42; *4* 0.53; *5* 1.11; *6* 1.76; *7* 2.50

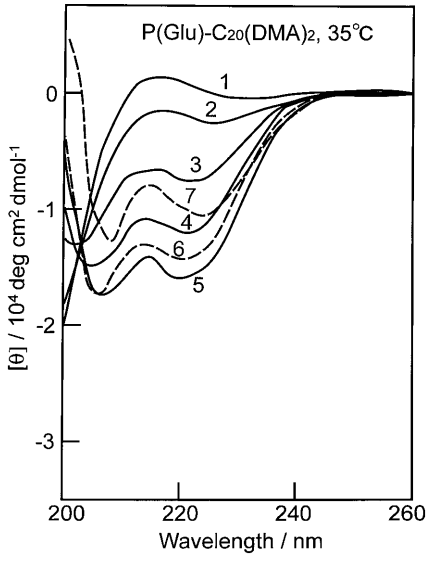

Fig. 5 CD spectra of P(Glu) in C20(DMA)$_2$ solutions at various $2C_s/C_p$ ratios at 35 °C. $2C_s/C_p$: *1* 0.00; *2* 0.20; *3* 0.42; *4* 0.53; *5* 0.75; *6* 1.11; *7* 2.50

a low $2C_s/C_p$ ratio, again indicating a single conformational change of P(Glu) from a random coil to an α-helix. The shapes of spectra 5 and 6 in Fig. 4, with double minima of equal intensity, are characteristic of the typical α-helix. A further increase in C20(DMA)$_2$ concentration induced a decrease in the intensity of the CD spectrum, which still has double minima, presumably owing to microphase separation where faint turbidity was visible. Clear temperature dependence is

observed in this system; the CD intensity is much lower at 35 than at 10 °C.

The CD spectra of P(Glu) in C20(TMA)$_2$ solutions at 10 and 35 °C are shown in Figs. 6 and 7, respectively. Figure 6 also includes spectra with double minima, indicating formation of the α-helix conformation as the surfactant concentration increases. These spectra also show an ambiguous isoelliptic point near 202 nm at a low $2C_s/C_p$ ratio (1–3 in Fig. 6 and 1–5 in Fig. 7), again indicating a single conformational change of P(Glu)

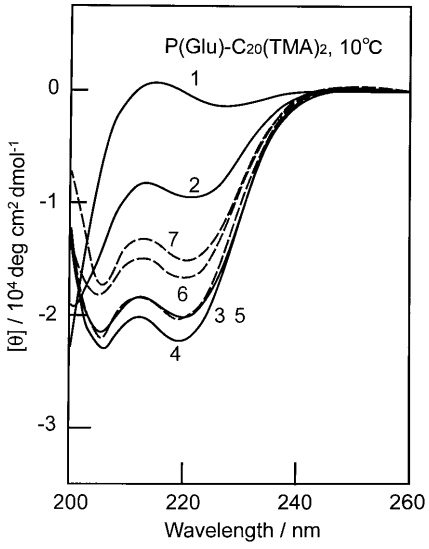

Fig. 6 CD spectra of P(Glu) in C20(TMA)$_2$ solutions at various $2C_s/C_p$ ratios at 10 °C. $2C_s/C_p$: *1* 0.00; *2* 0.20; *3* 0.42; *4* 0.53; *5* 0.64; *6* 1.11; *7* 2.50

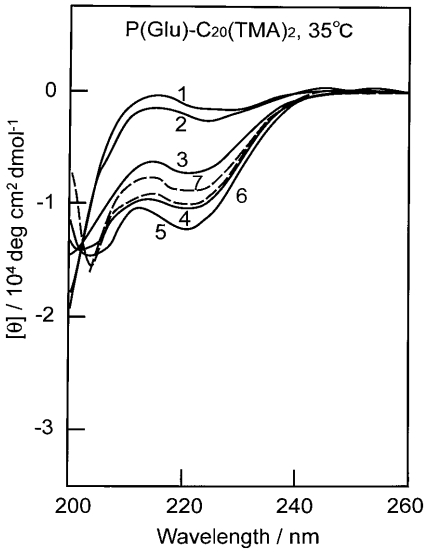

Fig. 7 CD spectra of P(Glu) in C20(TMA)$_2$ solutions at various $2C_s/C_p$ ratios at 35 °C. $2C_s/C_p$: *1* 0.00; *2* 0.20; *3* 0.42; *4* 0.53; *5* 0.64; *6* 1.36; *7* 2.50

from a random coil to an α-helix. The shape of spectrum 4 in Fig. 6, with double minima of equal intensity, is characteristic of the typical α-helix; however, the final maximum intensity is very low. A further increase in the $2C_s/C_p$ ratio induced a decrease in the intensity of the CD spectrum, which still has double minima, presumably owing to microphase separation. Strong temperature dependence is observed in this mixed system. The spectrum at 35 °C in Fig. 7 no longer shows the complete α-helix. Regular alkyltrimethylammonium surfactants have a reduced ability to induce the α-helix of P(Glu). Maeda et al. [10] observed a typical P(Glu) α-helix in dodecyltrimethylammonium surfactant solutions, but Hayakawa et al. [20] and Liu et al. [22] were not successful in finding the α-helix conformation of P(Glu) at concentrations below the onset of turbidity, using the same surfactant or 12-hydroxydodecyltrimethylammonium. The maximum CD intensity at 222 nm observed by Maeda et al. was −23,000, which is smaller than that found in P(Glu) in other alkylammoniums without a methyl group [19, 22], or with methyl and dimethyl groups [10, 20]. No α-helix has been reported in hexadecyltrimethylammonium solutions. Considering these observations, C20(TMA)$_2$ seems to be more suitable for inducing the α-helix of P(Glu) than regular surfactants with a TMA head group.

Concentration dependence

The dependence of the conformational change of P(Glu) on the equivalent ratio ($2C_s/C_p$) of the amphiphile to P(Glu) is shown for three amphiphiles at 10–35 °C in Fig. 8. The ellipticity at 222 nm, $[\theta]_{222}$, is used as a measure of the α-helix content in P(Glu). All three amphiphiles show a sharp decrease in $[\theta]_{222}$ below a ratio of 0.53, indicating α-helix formation as the amphiphile binding increases where the isoelliptic point is observed in their CD spectra.

Above a ratio of 0.53, the curves for the three amphiphiles separate into three directions. C20(MA)$_2$ shows a progressive decrease in $[\theta]_{222}$. The CD spectra in Figs. 1, 2, and 3 show a decrease in the intensity of the minimum at the shorter wavelength of 208 nm and a CD spectrum with a single minimum in this region of $2C_s/C_p$ is reached. $[\theta]_{222}$ reaches a minimum at $2C_s/C_p = 1.8$. The conformational change in this concentration range can be ascribed to the formation of helical aggregates [10, 20]. The plots of $[\theta]_{222}$ versus the $2C_s/C_p$ ratio for C20(DMA)$_2$ are nearly constant at 20 °C, decrease very slowly at 10 °C, and increase slowly at 35 °C above $2C_s/C_p = 0.53$, where the CD spectra do not show an isoelliptic point as shown in Figs. 4 and 5. At 35 °C, the curve does not reach a CD intensity of −20,000 and the spectrum change (5–7) in Fig. 5 at $2C_s/C_p > 0.53$

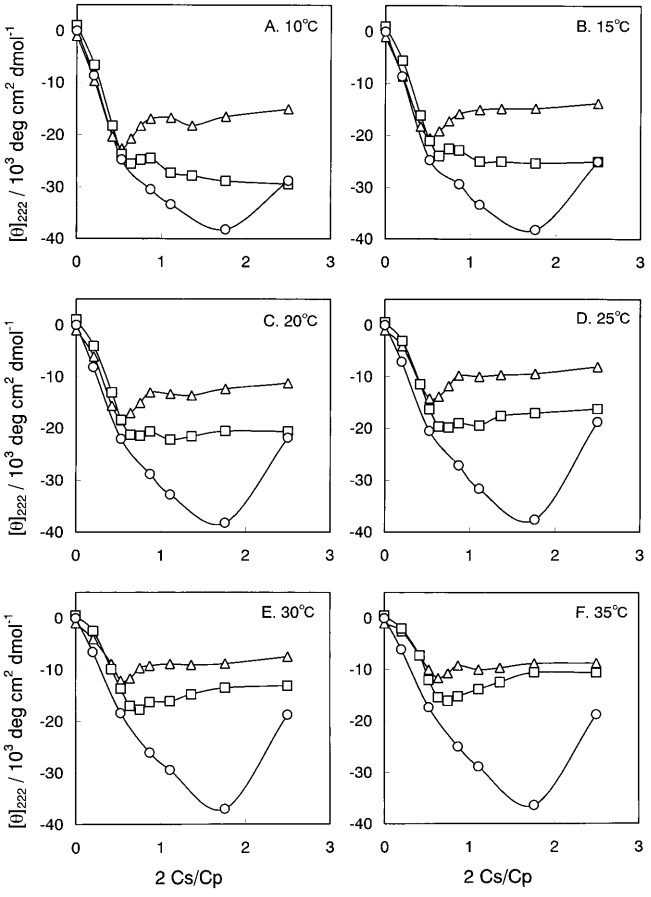

Fig. 8 Dependence of the residue ellipticity at 222 nm on the $2C_s/C_p$ ratio at various temperatures. $C20(MA)_2$ (*circles*), $C20(DMA)_2$ (*squares*), $C20(TMA)_2$ (*triangles*)

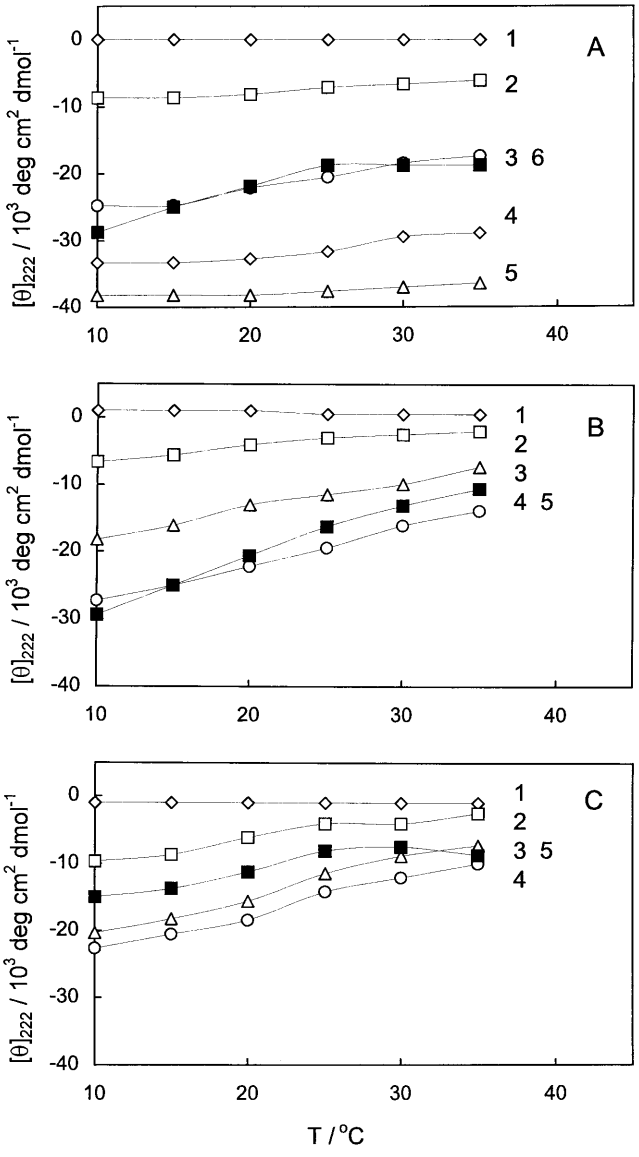

Fig. 9A–C Dependence of the residue ellipticity at 222 nm on solution temperature. **A** $C20(MA)_2$, $2C_s/C_p$: *1* 0.00; *2* 0.20; *3* 0.53; *4* 1.11; *5* 1.76; *6* 2.50 (*filled squares*); **B** $C20(DMA)_2$, $2C_s/C_p$: *1* 0.00; *2* 0.20; *3* 0.42; *4* 1.11; *5* 2.50 (*filled squares*); **C** $C20(TMA)_2$, $2C_s/C_p$: *1* 0.00; *2* 0.20; *3* 0.42; *4* 0.53; *5* 2.50 (*filled squares*)

suggests an increase in the random coil conformation, because the minimum is still observed at a shorter wavelength. At 10 °C, the CD intensity at 222 nm reaches −30,000 and the spectrum shows the typical α-helix conformation at $2C_s/C_p = 1.11$; subsequently the CD intensity at the minimum at the shorter wavelength slightly decreases, indicating a stable α-helix conformation over this range of $C20(DMA)_2$ concentrations. $[\theta]_{222}$ for $C20(TMA)_2$ increases slightly above $2C_s/C_p = 0.53$ and quickly becomes constant at $2C_s/C_p > 0.87$. A typical α-helix at the minimum at 10 °C is shown in Fig. 6, but P(Glu) does not form a typical α-helix at 35 °C, as indicated in Figure 7.

Temperature dependence

The CD spectra of P(Glu) in the three amphiphiles vary with the solution temperature. The temperature dependence of $[\theta]_{222}$ is plotted for three amphiphiles in Fig. 9. The strong α-helix producer $C20(MA)_2$ shows little change between 10 and 35 °C at $2C_s/C_p < 1.76$, suggesting a tight α-helix conformation in $C20(MA)_2$ solutions and little perturbation by temperature. $[\theta]_{222}$ show a greater change between 15 and 30 °C at $2C_s/C_p = 0.53$, corresponding to the midpoint in the conformational change from a random coil to an α-helix. This may be ascribed to large fluctuation at the midpoint of the cooperative phenomena [23, 24]. The solid marks correspond to the CD signal at the highest $2C_s/C_p$ ratio, where the CD intensity decreases owing to microphase separation. High temperatures accelerate α-helix aggregation.

Table 1 Conformations of poly(L-glutamic acid) [P(Glu)] in various amphiphile solutions. *n* and 2*n* in C*n* and C2*n* indicate the number of carbons in the alkyl chain. *A*: $-^{+}NH_3$; *MA*: $-^{+}NH_2(CH_3)$; *DMA*: $-^{+}NH(CH_3)_2$; *TMA*: $-^{+}N(CH_3)_3$; HO: ω-hydroxyl group

Surfactant	$n=10$	$n=11$	$n=12$	$n=14$	Reference
C2*n*$(MA)_2Cl_2$	Helix Aggregate				This work
C2*n*$(DMA)_2Cl_2$	Helix				This work
C2*n*$(TMA)_2Cl_2$	Helix				This work
C*n*TMACl			Helix Coil		[10] [20, 21]
C*n*DMACl			Helix Aggregate		[10, 20]
C*n*MACl			Helix Aggregate		[10]
C*n*ACl	Helix		Helix Aggregate	Helix	[19, 21] [10]
HOC*n*TMACl		Coil	Coil		[20]
HOC*n*DMACl		Coil	Helix Aggregate		[20]
HOC*n*MACl		Helix Aggregate	Helix Aggregate		[20]

$C20(DMA)_2$ and $C20(TMA)_2$ solutions showed a larger temperature dependence of $[\theta]_{222}$. A high temperature induced the random-coil conformation of P(Glu). Since the intermediate conformation between the random coil and the α-helix fluctuated more, the ratio of the two conformations may be temperature sensitive. Both amphiphile ions induced no α-helix aggregation with a single minimum, as described before.

Comparison with the results for other cationic amphiphiles

The conformations of P(Glu) in various ammonium amphiphile solutions are listed in Table 1, where C*n* indicates an alkyl chain with *n* carbons and HO indicates the ω-hydroxyl group in the amphiphile chain. The hydrophile–lipophile balance of $C20(RA)_2$ is thought to correspond to that of C10RA and is classified as a chain length of $n=10$. $C20(RA)_2$ induces an α-helix in P(Glu), whereas surfactants with an alkylammonium head group induce no α-helix at $n=10$. Only C10ACl has been found to induce an α-helix in P(Glu) [10, 19]. The fact that HOC12DMACl with an $n=12$ alkyl chain induced an α-helix, but HOC11DMACl with $n=11$ did not, indicates that amphiphiles with a longer alkyl chain are preferable for inducing the α-helix in P(Glu).

Maeda et al. [10] found that C12TMACl induced an α-helix in P(Glu) and showed a weaker interaction than C12DACl and C12DDACl, whereas Liu et al. [20] and Hayakawa et al. [22] did not succeed in finding an α-helix of P(Glu) in surfactants with a TMA head group. We measured the CD spectrum of P(Glu) in C16TMACl solutions and did not find an α-helix at concentrations below that of phase separation. These findings suggest steric hindrance in the compact α-helix conformation of

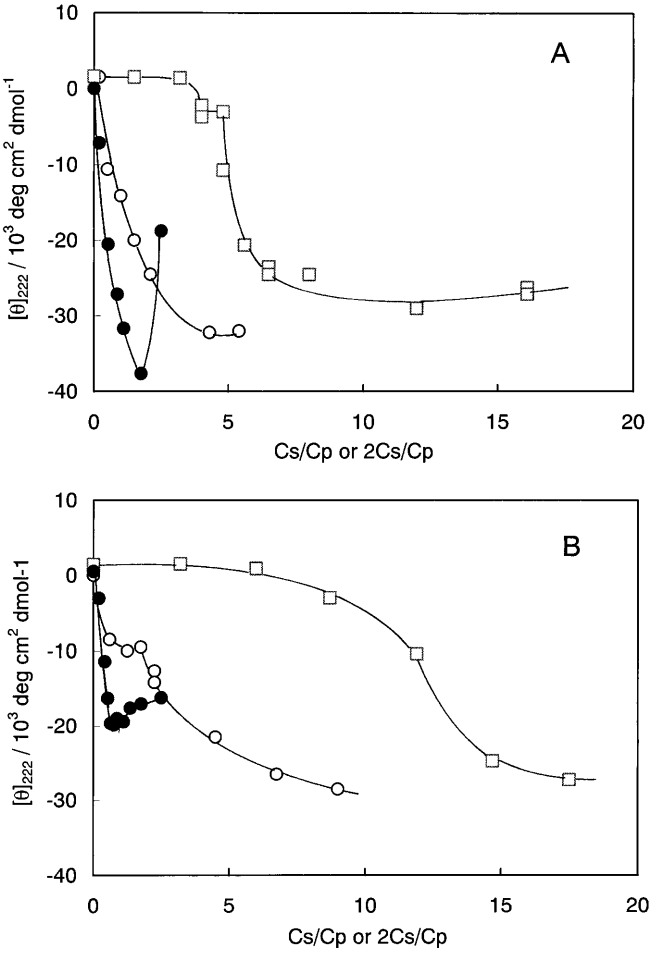

Fig. 10A,B Comparison of the residue ellipticity at 222 nm versus the $2C_s/C_p$ ratio for different amphiphile structures. **A** $C20(MA)_2$ (*filled circles*), C12MACl (*open circles*), HOC12MACl (*open squares*); **B** $C20(DMA)_2$ (*filled circles*), C12DMACl (*open circles*), HOC12DMACl (*open squares*)

P(Glu) by large head groups [25]. Maeda et al. found the order of the IR intensity of the asymmetric stretching of the $-COO^-$ group to be C12TMACl > C12ACl > C12DMACl in the solid complex of P(Glu) with these surfactant cations; a characteristic band for proton transfer at 1,720 cm^{-1} appeared in the solid P(Glu)–C12DMA complex, and suggested proton transfer from the C12DMA head to the carboxylic group of P(Glu) [26]. This may also be one of the origins for weak α-helix induction of the TMA head group in P(Glu), because amphiphiles with a TMA head group do not transfer a proton to P(Glu).

The $C20(RA)_2$ amphiphiles used in this study correspond to a carbon chain length of $n = 10$ in the hydrophile–lipophile balance, but nevertheless induced an α-helix in P(Glu). This is ascribed to the strong interaction with P(Glu). $[\theta]_{222}$ is shown in Fig. 10 as a function of C_s/C_p for amphiphiles with a single head and $2C_s/C_p$ for $C20(RA)_2$ amphiphiles. $C20(RA)_2$ induced an α-helix in P(Glu) at very low concentrations, indicating a stronger interaction with P(Glu) than C12MACl, C12DMACl, HOC12DMACl, or HOC12DMACl. Note that the *x*-axis indicates the equivalent ratio of the amphiphile to P(Glu). Although the two cationic groups in $C20(RA)_2$ are quite separate, they work as a divalent cation and interact strongly with anionic P(Glu), since a divalent counterion interacts much more strongly with polyelectrolytes than does a monovalent counterion [27]. However, the conformational change of polypeptide was found at a much higher ratio of $2C_s/C_p$ for the copper(II)–poly(L-lysine) mixed system [28].

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