Cooperative Binding and the Conformation of Poly(L-Glutamic Acid) in Guanidinium Salts with an Alkanoylamidoalkyl Group†

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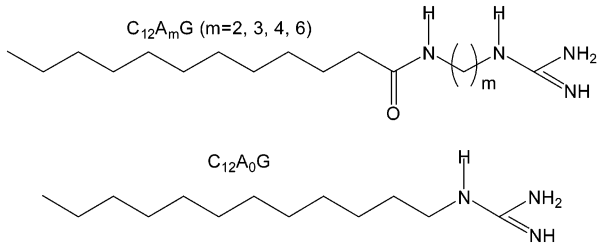
We investigated the change in the conformation of poly(L-glutamic acid) from a random coil to an α-helix in solutions of guanidinium salts with a dodecanoylamidoalkyl group, $C_{12}A_mG$ ($m = 0, 2, 3, 4, 6$), where A_m indicates a methylene spacer between the dodecanoyl group and guanidine and m is the number of methylene carbons. $C_{12}A_mG$s ($m = 0, 2, 3, 4$) induced an α-helix in P(Glu), but $C_{12}A_6G$ failed. Binding isotherms were constructed from the potentiometry of bound $C_{12}A_mG$ and cooperative binding was observed, as found in many regular surfactants. All $C_{12}A_mG$ gave a similar cooperative parameter and overall binding constants of comparative order (113×10^3 to 255×10^3). In contrast to the methylene group in regular surfactants, the methylene spacer in $C_{12}A_mG$ had a minor effect on hydrophobic interactions. $C_{12}A_0G$ showed the strongest cooperative binding and effect on the formation of the α-helix in P(Glu). The methylene spacer interfered with $C_{12}A_mG$ binding and α-helix formation in P(Glu).

Introduction

Guanidine hydrochloride and its analogues are very effective protein-denaturing agents. Polypeptide chains devoid of cross-links usually assume a random-coil conformation in 6 M guanidine hydrochloride, as evidenced by the physical properties of the solution, such as viscosity and optical rotatory spectra. Although the mechanism of action of the denaturants is not fully understood, it is evident that they disrupt noncovalent interactions in protein molecules. Many studies have examined denaturation, including the deactivation and unfolding of various enzymes and proteins induced by guanidinium salts.[1−5] Both ionic and nonionic surfactants denature proteins. Of these, sodium dodecyl sulfate is the most widely studied. Surfactants produce considerable conformational change at low concentrations, but the resulting conformation is still ordered and not randomly coiled. Tanford[6] treated the theoretical aspects of these interactions, and guanidinium salts with a hydrophobic tail are predicted to be strong denaturants that do not induce an ordered conformation in proteins. Nonspecific membrane permeability was almost completely inhibited by 0.1 mM octylguanidine hydrochloride, an amphiphilic cation, but not by hexylguanidine.[7] Mitamura et al.[8] developed a new cationic surfactant series of (dodecanoylamidoalkyl)guanidine hydrochlorides ($C_{12}A_mG$), in which the alkyl group (called a methylene spacer) consists of an ethyl ($m = 2$), propyl ($m = 3$), butyl ($m = 4$), or hexyl ($m = 6$) group plus dodecanoylguanidine hydrochloride ($C_{12}A_0G$). They found that the effect on skin or hair depends on the size of the methylene spacer. Of these compounds, $C_{12}A_4G$ reduced the brushing stress in running water and showed a high surface moisture coefficient in comparison with a conditioner containing octadecyltrimethylammonium, whereas $C_{12}A_0G$ stimulates skin. The strong dependence of the properties on the hydrophobic portion of the guanidinium salt motivated us to study the interaction of these salts with anionic homopolypeptides.



This paper reports the conformation of poly(L-glutamic acid) [P(Glu)] in solutions of $C_{12}A_mG$ and the binding behavior of the amphiphilic guanidinium salts to P(Glu) and discusses the dependence of the conformation of P(Glu) and the degree of binding on the structure of the amphiphilic guanidinium salts.

Experimental Section

Materials. Poly(L-glutamic acid) [P(Glu), MW > 8000] was purchased from Peptide Institute, Osaka, Japan. Amphiphilic guanidine hydrochlorides, $C_{12}A_mG$ ($m = 0, 2, 3, 4, 6$), were a gift from Lion Corporation; their synthesis has been reported elsewhere.[8] P(Glu) solutions in 2 M NaCl were repeatedly dialyzed against deionized water until no chloride was detected by silver nitrate. The stock solution pH was adjusted to 8 with sodium hydroxide and the ionic concentration was determined by colloid titration with poly(diallyldimethylammonium chloride).

Solution Preparation and CD and FTIR Measurements. Mixed solutions of P(Glu) and $C_{12}A_mG$ were kept at 25 °C for 1 night and the circular dichroic spectra were recorded on a Jasco J-500 spectropolarimeter. The temperature of the cell

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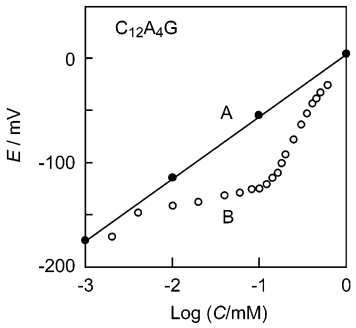

Figure 1. Potentiograph of $C_{12}A_6G$ in the absence (A) and presence (B) of P(Glu).

holder was kept at 25 °C by circulating thermoregulated water. P(Glu) concentration was expressed based on the monomeric residue. The pH of test solutions varied between 9.0 and 9.5. FTIR spectra were recorded on a Jasco FT/IR-5300.

Binding Isotherms. The equilibrium concentration of unbound $C_{12}A_mG$ was determined with potentiometry. The electrode membrane responsive to the $C_{12}A_mG$ cation was made from a mixture of partially sulfonated PVC and Elvaloy 742, a polymerized plasticizer (Du Pont) in a 2:3 ratio by weight. The partially sulfonated PVC was synthesized by the copolymerization of vinyl chloride and 2-acrylamido-2-methylpropane sulfonic acid at a molar ratio of 130:1. Since both of the membrane constituents are polymeric, the components will not dissolve in aqueous solutions. The electrode with this membrane has the cell formula shown.

Ag/AgCl electrode	agar salt bridge	reference solution	PVC membrane	test solution	agar salt bridge	Ag/AgCl electrode

This concentration cell exhibits an excellent Nernstian response in the absence of P(Glu), as illustrated by the straight line A in Figure 1. The decade slopes observed were 58−60 mV for $C_{12}A_mG$ ($m = 0$, 2, 3, 4, 6). In the presence of P(Glu), deviation from curve A was observed (curve B in Figure 1), suggesting that some of the surfactant is bound to the polymer. From the deviation, a binding isotherm is constructed following the usual method.[9]

Results and Discussion

Binding Isotherms. Figure 2 shows the binding isotherms of guanidinium salts by P(Glu), where C_f is the concentration of the unbound surfactant and $y = (C_S - C_f)/C_P$. C_S is the total concentration of the surfactant and C_P is the concentration of polymer in the glutamic acid unit, respectively. Binding isotherms were plotted, as shown in Figure 2. $C_{12}A_mG$ binding occurs at $y > 1$ without precipitation or turbidity. Some turbidity was observed at $y > 1.3$ for $C_{12}A_0G$, $y > 1.4$ for $C_{12}A_2G$ and $C_{12}A_4G$, and $y > 1.0$ for $C_{12}A_6G$. Most mixtures of polyelectrolyte and a surfactant of opposite charge form a precipitate near $y = 1$. These binding isotherms are characteristic of guanidinium salts.

At low $C_{12}A_mG$ concentrations, the binding isotherms reflect the features of cooperative binding of the surfactant by P(Glu), as observed in many systems consisting of a polyelectrolyte and a surfactant of opposite charge. The features of the cooperative binding include a sudden onset of binding and saturation within a narrow surfactant concentration in the binding isotherms. The binding of $C_{12}A_0G$ to P(Glu) is stronger than that of the other surfactants. These binding isotherms were

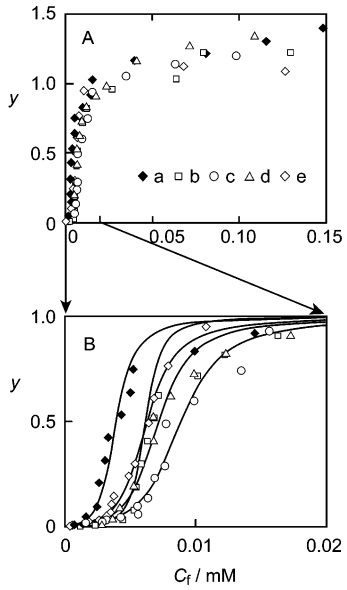

Figure 2. Binding isotherms of $C_{12}A_mG$: (a) $m = 0$, (b) $m = 2$, (c) $m = 3$, (d) $m = 4$, (e) $m = 6$. Panel B indicates the extended form at concentration C_f below 0.02 mM and the simulated curves by eq 1.

TABLE 1: Cooperative Binding Parameters and Average Cluster Size

surfactant	cmc (mM)	uK (mM^{-1})	u	m at $y = 0.5$
$C_{12}A_0G$	6.0	255	30	6.5
$C_{12}A_2G$	8.6	145	33	6.7
$C_{12}A_3G$	7.2	113	28	6.3
$C_{12}A_4G$	6.2	140	30	6.5
$C_{12}A_6G$	3.8	160	30	6.5

analyzed by the Satake−Yang equation:[10]

$$y = \frac{1}{2}\left\{1 - \frac{1 - s}{\sqrt{(1 - s)^2 + 4s/u}}\right\} \qquad (1)$$

where s is defined by uKC_f and equals a reduced concentration [$C_f/(C_f$ at $y = 0.5)$] and u measures cooperativity in surfactant binding. Physically, K is the intrinsic binding constant for an isolated binding site, and u is derived from the interaction energy between neighboring bound surfactants:

$$u = \exp(2w/kT) \qquad w = E_{OS} - (E_{SS} + E_{OO})/2 \qquad (2)$$

where w is the exchange energy between an empty site and a surfactant-bound site, as defined by the second equation, k is the Boltzmann constant, T is the Kelvin temperature, and E_{ij} is the paired interaction energy between sites i and j. The sites O and S correspond to an empty site and a site occupied by a surfactant ion, respectively. Therefore, u strongly depends on the hydrophobic property of the bound surfactants.

Simulations were carried out by choosing parameters to fit the lower half of the binding isotherm. Table 1 lists the values of binding affinity, uK, that give the best fit of the calculated binding isotherm (the solid lines in Figure 2) to the experimental ones. The observed order of the overall binding affinity, uK, is $C_{12}A_0G > C_{12}A_6G > C_{12}A_2G \sim C_{12}A_4G > C_{12}A_3G$. The order of uK for surfactant binding of opposite charge to a polyelectrolyte increases for surfactants with longer hydrophobic chains.[11,12] The cooperative parameter u, which includes a large uncertainty of 20%, is almost constant for these surfactants, indicating a similar hydrophobic interaction between bound $C_{12}A_mG$ ions. This may be ascribed to the fact that the hydrophobic interaction between $C_{12}A_mG$ molecules depends

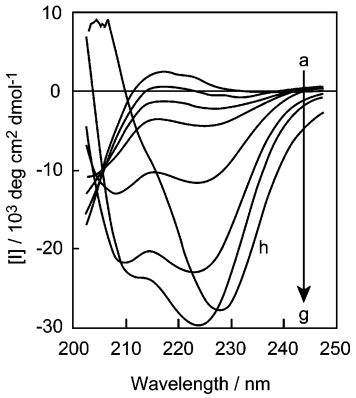

Figure 3. CD spectra of P(Glu) in $C_{12}A_0G$ solutions. Cs/Cp: (a) 0.0, (b) 0.22, (c) 0.44, (d) 0.78, (e) 1.11, (f) 1.39, (g) 1.67, (h) 1.94.

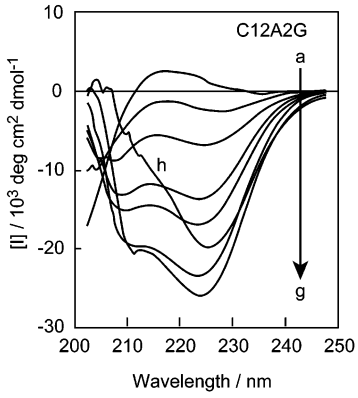

Figure 4. CD spectra of P(Glu) in $C_{12}A_2G$ solutions. Cs/Cp: (a) 0.0, (b) 1.11, (c) 1.39, (d) 1.67, (e) 1.94, (f) 2.22, (g) 2.50, (h) 2.78.

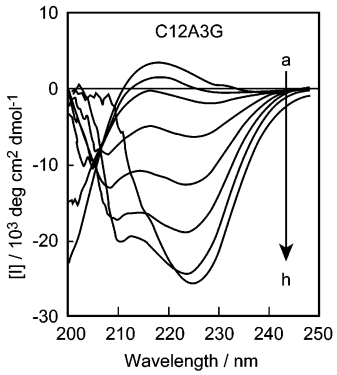

Figure 5. CD spectra of P(Glu) in $C_{12}A_3G$ solutions. Cs/Cp: (a) 0.0, (b) 0.26, (c) 0.99, (d) 1.19, (e) 1.39, (f) 1.59, (g) 1.79, (h) 1.98.

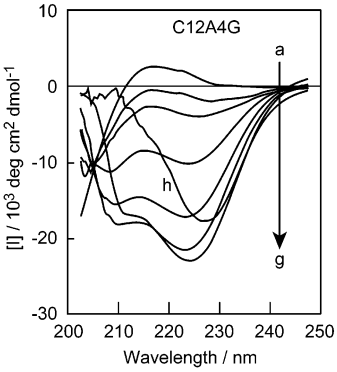

Figure 6. CD spectra of P(Glu) in $C_{12}A_4G$ solutions. Cs/Cp: (a) 0.0, (b) 0.78, (c) 1.11, (d) 1.39, (e) 1.67, (f) 1.94, (g) 2.22, (h) 2.50.

principally on the dodecyl chain. $C_{12}A_mG$ molecules have critical micelle concentrations (cmc) ranging from 3.8 to 8.0 mM, although the length of the methylene spacer differs by up to 6 methylene groups. The cmc of regular surfactants is 3 times lower when one methylene group is introduced in the hydrophobic chain. Liu et al.[13] estimated *uK* and *u* as 74.4 and 2117, 12.2 and 1587, and 1.2 and 257 for the binding of tetradecyl-, dodecyl-, and decylammonium by P(Glu), respectively. The much larger *uK* values may be ascribed to the multiple hydrogen bonds between $C_{12}A_mG$ and P(Glu) such as -CO···HN- and -CO···HO-. Our cooperative parameters are much lower than those for alkylammonium binding by P(Glu), suggesting a reduced hydrophobic interaction between bound $C_{12}A_mG$ ions because of strong interaction between $C_{12}A_mG$ and P(Glu).

According to the Satake−Yang treatment, the average size of a surfactant cluster, *m*, on a polyion is calculated from

$$m = \frac{2y(u-1)}{\sqrt{4y(1-y)(u-1)+1}-1} \qquad (3)$$

The calculated values at $y = 0.5$ are also given in Table 1. This cluster size depends only on the cooperative parameter *u* and the degree of surfactant binding *y*.

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 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, whereas a β-sheet gives one with a single minimum at 217 nm. Both ordered conformations result in CD spectra with a strong positive maximum below 200 nm, whereas the random coil conformation results in a CD spectrum with a strong minimum at 197 nm. Therefore, we can distinguish the conformation of polypeptides under various conditions from the CD spectrum.[14]

In the presence of $C_{12}A_0G$, the spectrum decreased at wavelengths from 205 to 250 nm (Figure 3), and spectrum f, with double minima at 208 and 223 nm, was obtained at 0.25 mM $C_{12}A_0G$; the minima had shifted to slightly longer wavelengths. A further increase in the $C_{12}A_0G$ concentration enhanced the CD intensity quickly at 223 nm and slowly at 208 nm and gave spectrum g. At 0.35 mM $C_{12}A_0G$, the CD minimum at 208 nm disappeared and a single minimum spectrum appeared (spectrum h). At 0.45 mM, we observed turbidity and no CD signal. 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.[15,16] Spectra a−d show an isoelliptic point at 204 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 e−g indicates a complex change, involving at least three states. The process of aggregation into the helical conformation may be responsible for the spectral change. We ascribed these spectral changes to the conformational change from a random coil to an α-helix and into helix aggregates, in various surfactant solutions.[16−19]

We observed similar CD spectral changes in solutions of other $C_{12}A_mG$s ($m = 2, 3, 4$) (Figures 4−7). The conformational changes of P(Glu) depend strongly on the hydrophilic head structure of the surfactants. Alkylammonium salts induce an α-helix in P(Glu), whereas alkylpyridinium salts and alkyltrimethylammonium salts do not.[13,20] A specific surfactant with alkylammonium groups at both ends induces an α-helix in P(Glu) and the effect decreases in the order methylammonium > dimethylammonium > trimethylammonium.[19] These facts

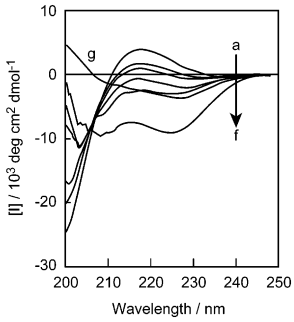

Figure 7. CD spectra of P(Glu) in $C_{12}A_6G$ solutions. Cs/Cp: (a) 0.0, (b) 0.16, (c) 0.32, (d) 0.52, (e) 0.64, (f) 0.79, (g) 0.99, (h) 1.39.

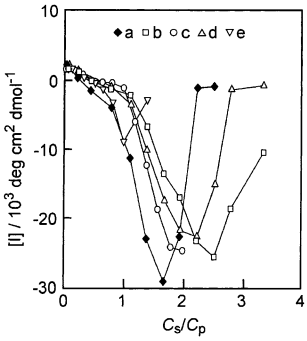

Figure 8. Dependence of CD intensity at 223 nm on the concentration of $C_{12}A_mG$: (a) $m = 0$, (b) $m = 2$, (c) $m = 3$, (d) $m = 4$, (e) $m = 6$.

indicate that surfactants with a large ionic headgroup have a weak ability to induce an α-helix in P(Glu). Since $C_{12}A_mGs$ induce an α-helix in P(Glu), we infer that the guanidinium group acts as an ionic headgroup in $C_{12}A_mG$ and that it is a similar size to the dimethylammonium head.

Dependence of CD Intensity on $C_{12}A_mG$ Concentration. We observed different concentration dependence and extreme intensity at 223 nm. The extreme intensity decreased as the methylene spacer was lengthened: it was 30 000 for $C_{12}A_0G$, 26 000 for $C_{12}A_2G$, 25 500 for $C_{12}A_3G$, 23 000 for $C_{12}A_4G$, and 9000 for $C_{12}A_6G$. The CD spectrum in $C_{12}A_6G$ solution had double minima at 0.25 mM, but it showed very weak CD intensity and did not correspond to a complete P(Glu) α-helix (Figure 7).

The CD intensity at 223 nm is plotted as a function of $C_{12}A_mG$ concentration in Figure 8. The molar ellipticity decreases as the $C_{12}A_mG$ concentration increases. A further increase in $C_{12}A_mG$ induces the increase in ellipticity, due to phase separation. The order in which an α-helix is induced at a lower concentration is $C_{12}A_0G > C_{12}A_3G > C_{12}A_4G > C_{12}$ A_2G. $C_{12}A_6G$ starts to induce an α-helix in P(Glu), but phase separation occurs before a complete α-helix forms. The longer the methylene spacer, the more hydrophobic $C_{12}A_mG$ is. Regular surfactants with a longer hydrophobic chain induce an ordered conformation in ionic polypeptides of opposite charge at a lower surfactant concentration, corresponding to the order of surfactant binding.[16,18,21,22] For $C_{12}A_mG$, the order of surfactant binding to P(Glu) is $C_{12}A_0G > C_{12}A_6G > C_{12}A_2G \sim C_{12}A_4G > C_{12}A_3G$ (Table 1). The effect of $C_{12}A_mG$ on the conformation of P(Glu) does not correspond to the binding behavior.

To examine this difference in the order of surfactant binding and induction of the α-helix, the CD intensity at 223 nm is plotted against the degree of $C_{12}A_mG$ binding to P(Glu) in Figure 9. With increasing degree of surfactant binding, the molar ellipticity decreases very slowly at $y < 1$ and sharply at $y > 1$

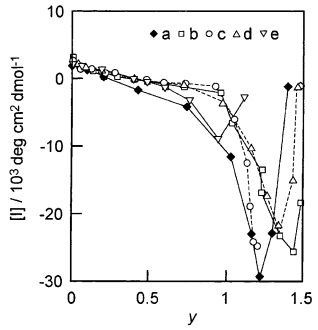

Figure 9. Dependence of CD intensity at 223 nm on the degree of $C_{12}A_mG$ binding: (a) $m = 0$, (b) $m = 2$, (c) $m = 3$, (d) $m = 4$, (e) $m = 6$.

and finally increases for the sake of phase separation of the P(Glu)−$C_{12}A_mG$ complexes. Again, $C_{12}A_0G$ has the strongest effect in producing a sharp decrease, but other $C_{12}A_mGs$ ($m = 2, 3, 4$) have a comparable effect in inducing an α-helix in P(Glu). In solutions of sodium alkanesulfonates with different chain lengths, the cationic polypeptide poly(L-ornithine) induced the α-helix conformation at a lower degree of surfactant binding for surfactants with longer hydrophobic chains.[16] This was ascribed to formation of a larger surfactant cluster in the polypeptide domain and a less hydrophobic environment due to the longer chain of the surfactant.[23] In our P(Glu)−$C_{12}A_mG$ system, each $C_{12}A_mG$ has a dodecyl group, but the lengths of the methylene spacer differ and each $C_{12}A_mG$ ($m = 2, 3, 4$) is expected to be more hydrophobic than $C_{12}A_0G$.

Satake and Yang[10] calculated that a stable α-helix conformation needs a surfactant cluster of at least 7−8 surfactant ions, which corresponds to two turns in the α-helix according to an investigation of surfactant binding and the conformation of poly(L-ornithine) in solutions of sodium decyl sulfate. The cluster size of 6−7 at $y = 0.5$ in Table 1 is too small to induce an α-helix in P(Glu). Equation 3 predicts that the cluster size is 8, 10, 14, and 22 at $u = 30$ and $y = 0.6$, 0.7, 0.8, and 0.9, respectively. This suggests that an α-helix could be induced in P(Glu) at $y > 0.6$. In fact, the induction of an α-helix occurs at $y > 1$ in the $C_{12}A_mG$−P(Glu) system, suggesting a specific interaction of $C_{12}A_mG$ with P(Glu) as discussed in the following paragraph.

We observed no precipitation at $y = 1$ and complete precipitation at $y \sim 1.5$. These facts suggest that the $C_{12}A_mG$−P(Glu) complex is partly surrounded by hydrophilic groups at $y = 1$. This difference in the behavior of $C_{12}A_mG$ and regular surfactants suggests a specific interaction of $C_{12}A_mG$ with P(Glu). $C_{12}A_mG$ possesses hydrogen-bonding groups, such as −CO, ═NH, and $-NH_2$, which can form hydrogen bonds with P(Glu), as well as with each other. FTIR measurements indicate disappearance of sharp amide bands around 1500∼1700 cm^{-1} and a band by N−H bond at 3279 cm^{-1} of $C_{12}A_4G$ solid in $C_{12}A_4G$−P(Glu) complex, while the bands at 2920 and 2851 cm^{-1} remain the sharp and strong alkyl chain band in the complex. These facts suggest an interaction of amide and amino group with P(Glu). Hydrogen bonding between $C_{12}A_mG$ and the P(Glu) amide group prevents formation of the P(Glu) α-helix. Every $C_{12}A_mG$ except $C_{12}A_0G$ has a peptide bond. Since $C_{12}A_0G$ lacks an amide bond as well as methylene spacer, it forms not a hydrogen bond with the amide group of P(Glu) but a hydrophobic environment around P(Glu) main chain, and this causes less interference in inducing an α-helix in P(Glu). Therefore, the methylene spacer affects both the conformation

of P(Glu) and the binding of $C_{12}A_mG$ to P(Glu), and may be responsible for its weak effect on human skin.

Conclusions

This study proved that $C_{12}A_mG$ binds strongly to P(Glu), presumably due to the double hydrogen bonds between the guanidinium head and the carboxyl group of P(Glu). Moreover, the methylene spacer between the acyl group and the guanidinium ion affects both $C_{12}A_mG$ binding and formation of the α-helix in P(Glu).

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