

Complexes of Poly(L-glutamic acid) with Various Polycations: Effect of the Structure of the Component Polymers

Koji ABE and Eishun TSUCHIDA

Department of Polymer Chemistry, Waseda University,
Tokyo 160, Japan.

(Received September 16, 1976)

ABSTRACT: Complexation abilities and the conformations of the complexes among different polymer chains are controlled by the structure of the component polymer chains. In the case of the combination of a strong and a weak polyelectrolyte, the composition of a complex in a unit molar ratio is determined mainly by the dissociation state of the weak polyelectrolyte which is presumably comparable to its conformation. The conformation of the complex (in this case, the conformational change is estimated from the stability change of the α -helical structure of poly(L-glutamic acid)) varies with the structure of the component polycations, for example, positions of active sites (pendant or integral type), distance between adjacent ionic sites, rigidity, hydrophobicity, and so on.

KEY WORDS Polyelectrolyte Complex / Poly(L-glutamic acid) / Polycation / Conformations / Cooperativity / Circular Dichroism /

Macromolecules in solution can interact with each other to make an interpolymer complex through various secondary binding forces; *i.e.*, Coulombic force, hydrogen bond, van der Waals force, and hydrophobic interaction. Only a few works¹⁻⁴ about such interpolymer complexation among artificial polymers have been published so far, in spite of its importance as a simple and suitable model for the assembly in biological systems.

The formation of an interpolymer complex is controlled by many factors; for example, the kind of interaction force, concentration, dissociation states of polyelectrolytes, ionic strength, solvent, temperature, and structures of the component polymers. In previous papers,⁵⁻⁷ the authors have shown how and why the factors mentioned above affect the interpolymer complexation. In the last paper,⁸ it was found that the α -helical structure is destabilized by complexation with more than a critical chain length of oligo(ethyleneimine) and their quaternary derivatives. Therefore, in this paper, the effects of the structure of a component polymer chain on complexation are discussed.

EXPERIMENTAL

Materials

Poly(L-glutamic acid)(Glu)_n. (Glu)_n was prepared by exchanging counter ions of a commercial poly(sodium-L-glutamate) ($\bar{M}_w=50000$) before use in a similar manner to the former paper.⁸

Poly(methacrylic acid)(MeAcrOH)_n, *Poly(acrylic acid)(AcrOH)_n*. Purified methacrylic acid and acrylic acid (distilled *in vacuo* twice, bp 63°C/12 mmHg and 52°C/20 mmHg, respectively) were polymerized with K₂S₂O₈ as an initiator in aqueous solution. The monomer concentration was 0.2 (mol/l) and the polymerization time was 5hr in nitrogen atmosphere at 50°C. The reaction products were reprecipitated twice into methanol/ethyl acetate. The molecular weights of the polymers were calculated from viscosity measurements,^{9,10} and \bar{M}_w of (MeAcrOH)_n and (AcrOH)_n were 6.8×10^4 and 4.9×10^4 , respectively.

Polycations. The structural formulas and intrinsic viscosities of the pendant and integral type polycations are listed in Table I. Integral type polycations, which had cationic sites on

Table I. Structures of polycationic polymers

Integral-type polycations			
$\left[\begin{array}{c} \text{CH}_3 \quad \text{Cl}^- \quad \text{CH}_3 \quad \text{Cl}^- \\ \quad \quad \quad \\ -\text{N}^+ - \text{R}_1 - \text{N}^+ - \text{R}_2 - \\ \quad \quad \quad \\ \text{CH}_3 \quad \text{CH}_3 \end{array} \right]_n$			
Abbreviations	R ₁	R ₂	[η]
(N ⁺ Me ₂ EtN ⁺ Me ₂ CH ₂ phCH ₂ ·Cl ⁻) _n	-(CH ₂) ₂ -	-CH ₂ phCH ₂ -	0.114 ^a
(N ⁺ Me ₂ (CH ₂) ₃ N ⁺ Me ₂ CH ₂ phCH ₂ ·Cl ⁻) _n	-(CH ₂) ₃ -	-CH ₂ phCH ₂ -	0.116 ^a
(N ⁺ Me ₂ (CH ₂) ₆ N ⁺ Me ₂ CH ₂ phCH ₂ ·Cl ⁻) _n	-(CH ₂) ₆ -	-CH ₂ phCH ₂ -	0.164 ^a
(N ⁺ Me ₂ CH ₂ phCH ₂ ·Cl ⁻) _n	-CH ₂ phCH ₂ -	-CH ₂ phCH ₂ -	0.167 ^a
(N ⁺ Me ₂ (CH ₂) ₄ ·Cl ⁻) _n	-(CH ₂) ₄ -	-(CH ₂) ₄ -	0.087 ^b
(N ⁺ Me ₂ (CH ₂) ₆ ·Cl ⁻) _n	-(CH ₂) ₆ -	-(CH ₂) ₆ -	0.123 ^b
(N ⁺ Me ₂ (CH ₂) ₈ ·Cl ⁻) _n	-(CH ₂) ₈ -	-(CH ₂) ₈ -	0.174 ^b
(N ⁺ Me ₂ (CH ₂) ₁₀ ·Cl ⁻) _n	-(CH ₂) ₁₀ -	-(CH ₂) ₁₀ -	0.093 ^b
Pendant-type polycations			
Abbreviations	Structure		[η]
(CH ₂ CHBzIN ⁺ Me ₃ ·Cl ⁻) _n	$\begin{array}{c} \uparrow \\ \text{CH}_2 \\ \\ \text{CH} - \text{C}_6\text{H}_4 - \text{CH}_2\text{N}^+(\text{CH}_3)_3 \\ \\ \uparrow \\ \text{Cl}^- \end{array}$		0.271 ^a
(CH ₂ OCHCH ₂ N ⁺ Me ₃ ·Cl ⁻) _n	$\begin{array}{c} \uparrow \\ \text{O} \\ \\ \text{CH} - \text{CH}_2 - \text{N}^+ - (\text{CH}_3)_3 \\ \\ \text{CH}_2 \\ \uparrow \\ \text{Cl}^- \end{array}$		0.345 ^a

^a 0.2-mol/l KCl aq soln at 25(±0.01)°C.

^b 0.4-mol/l KBr aq soln at 25(±0.01)°C.

their chain backbones, were prepared in a normal successive Menshutkin reaction, as described in a previous paper.¹¹ Pendant type polycations, which had cationic sites on their side chains, were prepared by a quaternization reaction of tertiary amines. Poly(vinylbenzyltrimethylammonium chloride)(CH₂CHBzIN⁺Me₃·Cl⁻)_n, was Dow Chemical ECR-34 purified by reprecipitation twice from methanol into acetone. Poly(oxyethyl-1-methylene-trimethyl ammonium chloride)(CH₂OCHCH₂N⁺Me₃·Cl⁻)_n, was prepared by polymer reaction of epichlorohydrine with trimethylamine and the reaction product was reprecipitated twice from methanol into acetone. Their degrees of quaternization were 79% and 100%, respectively, by Volhard's and elementary analyses.

Poly(4-vinylpyridine)(CH₂CH-4-Py)_n and Poly(2-vinylpyridine)(CH₂CH-2-Py)_n. They

were synthesized by the radical polymerizations of 4-vinylpyridine and 2-vinylpyridine, respectively, under the following conditions: [monomer]=2.0 mol/l, [AIBN]=0.05 mol/l, in methanol at 70°C for 6 hr under a nitrogen atmosphere. The products were poured into a large excess of ethyl ether and further purification was done by repeating this process. Their degrees of polymerization (\bar{P}_n) were 49 for (CH₂CH-4-Py)_n and 108 for (CH₂CH-2-Py)_n, respectively as determined by means of a vapor pressure osmometer in methanol.

Quaternized Oligo(ethyleneimine) H(H₂CN⁺Me₂·CH₂·Cl⁻)_nH. These were prepared in a similar manner to the previous paper.⁸

Measurement of Complexation

Circular dichroism (CD) and potentiometric titration were measured in a similar manner to the previous paper.⁸

RESULTS AND DISCUSSION

Polycations far longer than a critical chain length form stable complexes with poly(carboxylic acid)s such as poly(methacrylic acid)(MeAcrOH)_n, poly(acrylic acid)(AcrOH)_n, and poly(L-glutamic acid)(Glu)_n. The compositions of their complexes are changed mainly due to the dissociation states of poly(carboxylic acid)s when the polycations used are strong polyelectrolytes.* Figure 1 shows the potentiometric titration curves of the aqueous solution of (Glu)_n by that of a polycation, (N⁺Me₂(CH₂)₃N⁺Me₂CH₂phCH₂·Cl₂)_n. Differing from the neutralization titration of low molecular acids and bases, the changes of these titration curves are more indistinct, since such polymer-polymer complexation mechanism is an equilibrium composed of many steps. However, as soon as the complexation is completed, a steep decrease of pH is observed, because of the polycations (pH of the aqueous solution of the polycation used here,

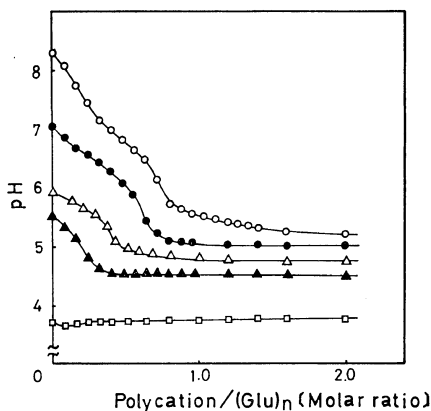


Figure 1. Potentiometric titrations of (Glu)_n by polycation at various degrees of dissociation of (Glu)_n: Initiated pH, ○, 8.3; ●, 7.0; △, 6.0; ▲, 5.5; □, 3.7; [(Glu)_n], 5.0 × 10⁻⁴ base mol/l; [(N⁺Me₂(CH₂)₃N⁺Me₂CH₂phCH₂·Cl₂)_n], 5.0 × 10⁻³ base mol/l; 25°C.

* In the system of (N⁺Me₂(CH₂)₃N⁺Me₂CH₂phCH₂·Cl₂)_n-(MeAcrOH)_n and -(AcrOH)_n, the compositions of the complexes are represented as:

$$\frac{[\text{Cationic sites of polycation}]}{[\text{Anionic sites of poly(carboxylic acid)}]} = \alpha' \quad (1)$$

where α' is a degree of dissociation of poly(carboxylic acid).⁶

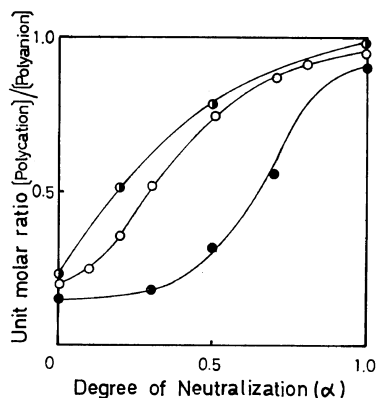


Figure 2. Relation between the compositions and the degrees of neutralization of poly(carboxylic acids): (N⁺Me₂(CH₂)₃N⁺Me₂CH₂phCH₂·Cl₂)_n-(MeAcrOH)_n, ○; (AcrOH)_n, ●; (Glu)_n, ●. The experimental conditions are similar to Figure 1.

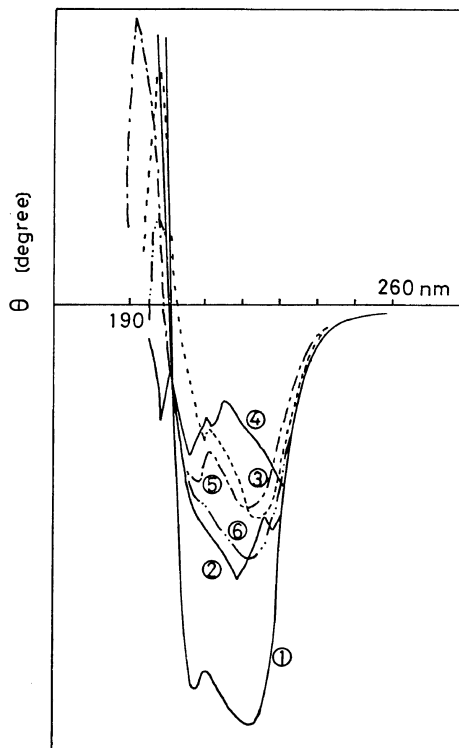


Figure 3. CD spectra of (Glu)_n-polycation complexes: (Glu)_n, ①; its complexes with (N⁺Me₂Et-N⁺Me₂CH₂phCH₂·Cl₂)_n, ②; (N⁺Me₂(CH₂)₃N⁺Me₂CH₂phCH₂·Cl₂)_n, ③; (N⁺Me₂CH₂phCH₂·Cl₂)_n, ④; (CH₂OCHCH₂N⁺Me₃·Cl₂)_n, ⑤; (CH₂CHBzlN⁺Me₃·Cl₂)_n, ⑥; [(Glu)_n] = [Polycation], 2.5 × 10⁻⁴ base mol/l; pH, 4.6; 22°C.

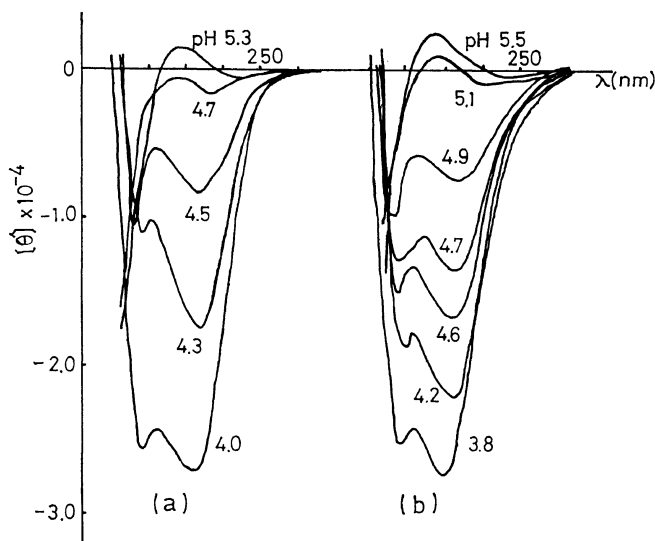


Figure 4. The change of the CD spectra of $(\text{Glu})_n^-$ polycation complexes: (a), $(\text{Glu})_n^- (\text{N}^+\text{Me}_2(\text{CH}_2)_4 \cdot \text{Cl}^-)_n$; (b), $(\text{Glu})_n^- (\text{N}^+\text{Me}_2(\text{CH}_2)_{10} \cdot \text{Cl}^-)_n$. The experimental conditions are similar to Figure 3.

5×10^{-4} base mol/l, is 4.2 at 25°C). So the compositions of the complexes are obtained from an inflection point of each titration curve. The relation between the composition of the complex (taken from Figure 1) and the degree α of neutralization of poly(carboxylic acid) is plotted in Figure 2. Compared with the $(\text{AcrOH})_n$ system the $(\text{MeAcrOH})_n$ and $(\text{Glu})_n$ systems show sigmoidal curves. These facts seem to correspond with their conformational changes; in other words, the discontinuous changes of their dissociation states induced by the conformational changes. While $(\text{AcrOH})_n$ dissociates successively with an increase of the degree of the neutralization, like a usual weak polyelectrolyte, dissociations of $(\text{MeAcrOH})_n$ and $(\text{Glu})_n$ are effectively restrained until α is nearly equal to 0.1 and 0.5, respectively, because of their specific conformations: *i.e.*, a rather rigid and compact structure, owing to the hydrophobic interaction among α -methyl groups along the $(\text{MeAcrOH})_n$ chain,¹² and the α -helical structure of $(\text{Glu})_n$. On the other hand, the differences among the structure of polycations influence the composition less effectively. The composition of the pendant type polycation— $(\text{Glu})_n$ complex is larger than that of the integral type polycation— $(\text{Glu})_n$ complex, but the features of the titration curves are not

essentially changed.

Figure 3 is the CD spectra of $(\text{Glu})_n$ and its complexes with various polycations at pH 4.5, and Figure 4 shows typical CD spectrum changes against pH change. A negative peak at 222 nm is shifted to longer wavelength and its magnitude is decreased, and the other peaks, a negative peak at 208 nm and a positive peak at 195 nm, are barely observable at their original positions. It is found that the α -helical structure of $(\text{Glu})_n$ is destabilized by complexation with polycations, while other oriented conformations different from the α -helical or β -form structure taken by complexation. In this paper, the oriented conformation contents of complexes are calculated from the shifted negative peaks and pH value at the midpoint of the transition (pH_t) is obtained as an inflection point of a $[\theta']$ vs. pH curve (see Figure 4). Integral-type polycations make the α -helical structure effectively unstable as a rule, but the degree of destabilization is extremely different depending on their structures, especially the interval between active sites, rigidity, and so on. On the contrary, pendant-type polycations influence the α -helical structure less effectively, probably owing to the flexibility of their backbones and/or to their configurational disorders.

Such oriented conformation is reformed to a more conformationally stable state, *i.e.*, random coil structure with a time-dependence of the order of hours because of the new bindings in intra-complex, and/or the correction of the distortions induced by complexation. Thus, the complex formed immediately after mixing seems to be in a non-equilibrium states. Moreover, their destructions are faster than those by the pendant-type polycations.

If the complexes are formed in an alkaline

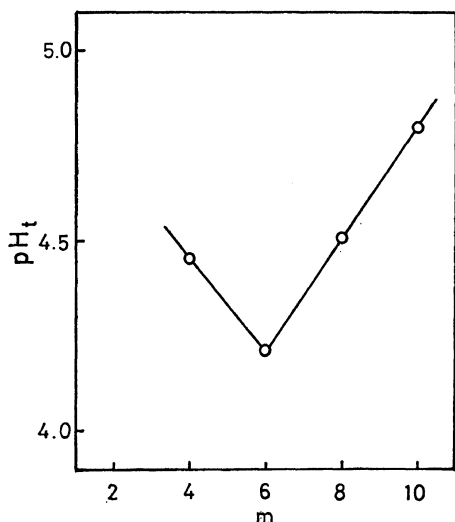


Figure 5. Dependence of the conformational transition pH (pH_t) on the number of methylene residues (m) between adjacent ionic sites of $(\text{N}^+\text{Me}_2(\text{CH}_2)_m \cdot \text{Cl}^-)_n$: $[(\text{Glu})_n] = [\text{Polycation}]$, 2.5×10^{-4} base mol/l; 22°C .

Table II. Conformational transition pH (pH_t), apparent dissociation constant ($\text{p}K_a$), and interaction parameter between adjacent ionic sites (n') of $(\text{Glu})_n(\text{N}^+\text{Me}_2(\text{CH}_2)_m \cdot \text{Cl}^-)_n$ complex

	Additions				
	None	$(\text{N}^+\text{Me}_2(\text{CH}_2)_m \cdot \text{Cl}^-)_n$			
		$m=4$	$m=6$	$m=8$	$m=10$
pH_t	6.57	4.45	4.21	4.51	4.80
$\text{p}K_a^a$	6.23	4.48	4.33	4.46	4.53
n'^a	1.6	1.1	0.75	1.1	1.4

^a $\text{p}K_a$ and n' are obtained from Henderson—Hasselbach equation: $[(\text{Glu})_n]$, 2.5×10^{-4} base mol/l; $[\text{Polycation}]$, 2.5×10^{-5} base mol/l; 22°C .

region and then the solutions are set in an acidic region, even at pH 4.5, they are all random coil structure, different from the results in Figure 3. It is found that the complexes which are formed under the condition of random coil structure are reformed to α -helical or other oriented conformations only with difficulty.

Figure 5 shows the relationship between pH_t and distance between two cationic sites on $(\text{N}^+\text{Me}_2(\text{CH}_2)_m \cdot \text{Cl}^-)_n$ type polycations (distance between two cationic sites is represented as the number of methylene residues between two cationic sites). Their pH_t , apparent dissociation constants ($\text{p}K_a$), and interaction parameters between adjacent carboxylic groups of $(\text{Glu})_n(n')$ calculated from the Henderson—Hasselbach equation⁵ are shown in Table II. pH_t , $\text{p}K_a$, and n' show a minimum at $m=6$. It is found that the interval of active sites has a very important

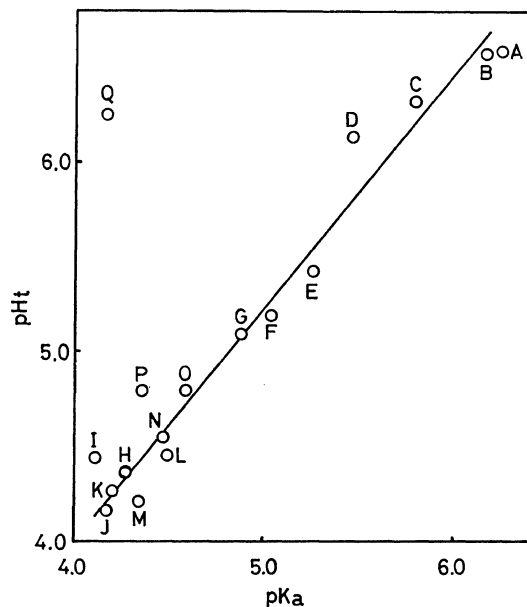


Figure 6. Relationship between conformational transition pH (pH_t) and apparent dissociation constants ($\text{p}K_a$) of $(\text{Glu})_n$ -polycation complexes A, $(\text{Glu})_n$; complexes with H $(\text{CH}_2\text{N}^+\text{Me}_2\text{CH}_2 \cdot \text{Cl}^-)_n$, B, $n=1$; C, $n=2$; D, $n=3$; E, $n=4$; F, $n=5$; G, $n=6$; complexes with $(\text{N}^+\text{Me}_2(\text{CH}_2)_m \text{N}^+\text{Me}_2\text{CH}_2\text{-phCH}_2 \cdot \text{Cl}^-)_n$, H, $m=2$; I, $m=3$; J, $m=6$; K, complex with $(\text{N}^+\text{Me}_2\text{CH}_2\text{PhCH}_2 \cdot \text{Cl}^-)_n$; complexes with $(\text{N}^+\text{Me}_2(\text{CH}_2)_m \cdot \text{Cl}^-)_n$; L, $m=4$; M, $m=6$; N, $m=8$; O, $m=10$; P, complex with $(\text{CH}_2\text{CHBzN}^+\text{Me}_3 \cdot \text{Cl}^-)_n$; Q, complex with $(\text{CH}_2\text{OCH}_2\text{N}^+\text{Me}_3 \cdot \text{Cl}^-)_n$.

role in forming complexes, since carboxylic groups along $(\text{Glu})_n$ chain are fixed around the rigid α -helical structure. $(\text{N}^+\text{Me}_2(\text{CH}_2)_6\cdot\text{Cl}^-)_n$ acts on carboxylic groups along the $(\text{Glu})_n$ chain as counter ions to make it easy for them to dissociate most effectively (both pK_a and n' are lower). Namely, $(\text{N}^+\text{Me}_2(\text{CH}_2)_6\cdot\text{Cl}^-)_n$ forms a complex with $(\text{Glu})_n$ most easily. As the Coulombic interaction is a long range force, other polycations can also interact with carboxylic groups of $(\text{Glu})_n$ but their destructive effects on the α -helical structure are weak. As a result, the decrease of pH_t seems to be caused by the decrease of pK_a , *i.e.*, the increase in facility of dissociation of carboxylic groups. Figure 6 is the relation between pH_t and pK_a of various complexes. pH_t is decreased in proportion to pK_a . Moreover, the results in this Figure can be divided into several classes: *i.e.*, oligocations, $-(\text{CH}_2)_m-$ type polycation, $-\text{CH}_2\text{phCH}_2-$ type polycation, and pendant-type polycation. Though pendant-type polycations have lower pK_a , their pH_t are not so low as pK_a , probably owing to the flexibility of their backbones and/or their configurational disorders. In the integral-type polycation systems, the effect of polycations on the destabilization of the α -helix is in the order of $-\text{CH}_2\text{phCH}_2->-(\text{CH}_2)_m->$ oligocations, because of the difference in their chain lengths, rigidities, and steric hindrances.

Table III shows salt effects on complexation. With increasing micro salt content, the difference of pH_t among various polycations becomes smaller and finally pH_t values become identical.

Table III. Salt effects on conformational transition pH (pH_t) of $(\text{Glu})_n-(\text{N}^+\text{Me}_2(\text{CH}_2)_m\cdot\text{Cl}^-)_n$ complexes

[NaCl]	Addition				
	None	$(\text{N}^+\text{Me}_2(\text{CH}_2)_m\cdot\text{Cl}^-)_n$			
		$m=4$	$m=6$	$m=8$	$m=10$
0	6.5 ₀	4.4 ₅	4.2 ₁	4.5 ₁	4.8 ₀
0.01 mol/l	5.5 ₅	4.6 ₃	4.5 ₅	4.7 ₄	4.9 ₈
0.1 mol/l	5.1 ₂	4.5 ₅	4.5 ₅	4.5 ₅	4.5 ₅
1.0 mol/l	4.8 ₁	4.8 ₁	4.8 ₁	4.8 ₁	4.8 ₁
0.1 ^a mol/l	4.6 ₀	4.6 ₀	4.6 ₀	4.6 ₀	4.6 ₀

^a 0.1, 0.1-mol/MgCl₂; $[(\text{Glu})_n]$, 2.5×10^{-4} base mol/l; [Polycation], 2.5×10^{-5} base mol/l; 22°C.

The α -helix of $(\text{Glu})_n$ is destabilized on adding micro salts,¹⁴ but at the same time the Coulombic force is also weakened by adding salt, so that the interaction force between $(\text{Glu})_n$ and polycations is weakened according to the amount of salt added. The influence of the structure of polycations on the stability of the α -helical structure becomes smaller and finally the complex is not formed, as for example in the salt concentration of more than 1.0-M NaCl or 0.1-M MgCl₂.

These complexes are expected to have different properties from the component polyelectrolytes. For example, the solubilities of $(\text{N}^+\text{Me}_2(\text{CH}_2)_{10}\cdot\text{Cl}^-)_n$ and $(\text{N}^+\text{Me}_2(\text{CH}_2)_6\cdot\text{Cl}^-)_n-(\text{Glu})_n$ complexes are shown in Figure 7. The complexes are soluble in the ternary solvent mixture.¹³ Such a ternary solvent mixture seems to dissolve the polyion complexes by the following mechanisms: microsalts weaken the Coulombic force and the organic solvent weakens the hydrophobic interaction. $(\text{N}^+\text{Me}_2(\text{CH}_2)_{10}\cdot\text{Cl}^-)_n$ is more hydrophobic due to more methylene residues than $(\text{N}^+\text{Me}_2(\text{CH}_2)_6\cdot\text{Cl}^-)_n$, so the part dissolving the complexes in the ternary solvent mixture is located in a more organic solvent-rich mixture. Moreover, their X-ray diffraction patterns are different from each other and from that of $(\text{Glu})_n$. It is found that the conformation and the physical properties of the complex are different from the structure and the property of the component polyelectrolytes, but reflects them partially.

Figure 8a shows the conformational change by complexation, and Figure 8b the pH depend-

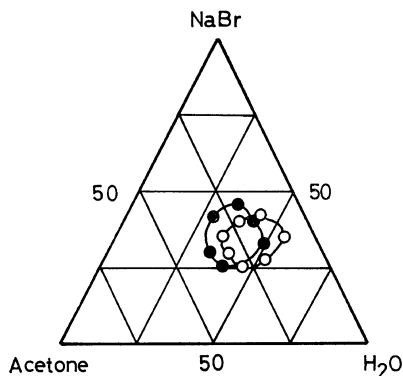


Figure 7. Solubilities of $(\text{Glu})_n-(\text{N}^+\text{Me}_2(\text{CH}_2)_m\cdot\text{Cl}^-)_n$ complexes: ○, $m=6$; ●, $m=10$.

Complexes of PGA with Polycations

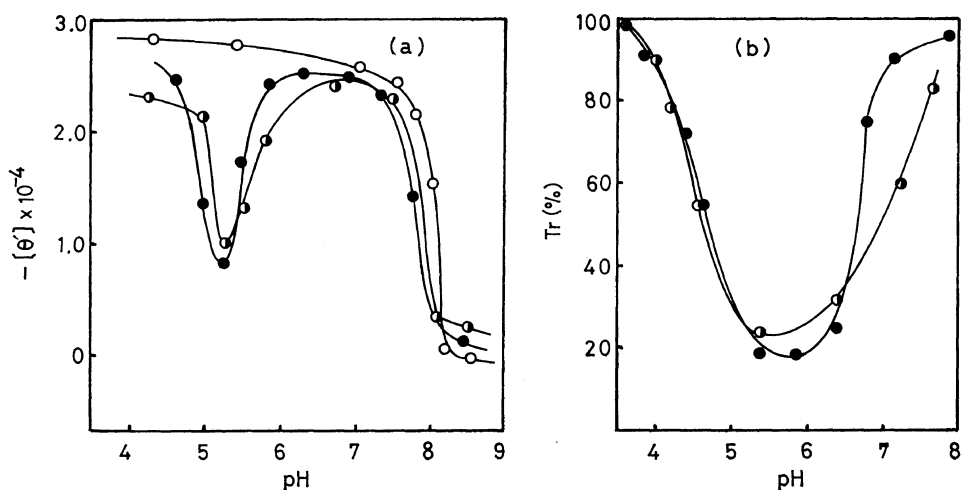


Figure 8. pH dependence of complexation in $(\text{Glu})_n-(\text{CH}_2\text{CHPy})_n$ complex: (a) helix content, $[(\text{Glu})_n]$, 2.5×10^{-4} base mol/l; (b) transmittancy, $[(\text{Glu})_n]$, 5×10^{-3} base mol/l; \circ , $(\text{Glu})_n$; \bullet , $(\text{Glu})_n-(\text{CH}_2\text{CH}-2-\text{Py})_n$; \bullet , $(\text{Glu})_n-(\text{CH}_2\text{CH}-4-\text{Py})_n$; $[(\text{Glu})_n]/[(\text{CH}_2\text{CHPy})_n]$, 1/1; in water—methanol (50 : 50); 22°C .

Table IV. Elementary analysis of $(\text{Glu})_n-(\text{CH}_2\text{CHPy})_n$ complexes at various pH^a

Sample	pH	C, %	H, %	N, %	C/N	$[(\text{CH}_2\text{CHPy})_n]/[(\text{Glu})_n]$
$(\text{Glu})_n-(\text{CH}_2\text{CH}-2-\text{Py})_n$	5.1	57.7	59.0	11.5	5.0	0.8
	6.1	59.9	5.86	11.6	5.2	1.0
	6.5	64.0	6.24	12.1	5.3	1.3
$(\text{Glu})_n-(\text{CH}_2\text{CH}-4-\text{Py})_n$	5.1	55.4	5.97	11.2	5.0	0.7
	5.7	59.0	6.10	11.6	5.1	0.9
	6.1	60.4	6.23	11.4	5.3	1.3

^a In water—methanol (50 : 50).

ence of transmittancy, which corresponds to the formation of the complex. As shown in Figure 8b, the complex is formed only in a suitable pH range, $4.5 < \text{pH} < 6.5$, and this fact coincides well with the destruction of the α -helical structure of $(\text{Glu})_n$ as shown in Figure 8a. The same phenomena are observed when the complexes are formed in an alkaline region and then the pH of the solutions are changed to an acidic region. The difference of the position of the active sites has no influence on the complexation in this case. Similar phenomena are observed in the system of $(\text{Glu})_n$ -oligo(ethyleneimine) in water—methanol.⁸ Considering that both polyelectrolytes are weak polyelectrolytes and that a certain number of active sites are necessary to form a complex, the complex is not formed until both component polyelectrolytes are of the

proper dissociation states: in low pH region, $(\text{CH}_2\text{CHPy})_n$ is almost protonated but $(\text{Glu})_n$ is scarcely dissociated, so that they can interact only very weakly through ion—dipole or ion—ion interaction, while, in a higher pH region, $(\text{Glu})_n$ is almost dissociated $(\text{CH}_2\text{CHPy})_n$ is scarcely protonated, so that they can hardly interact; but in a suitable pH region, both polyelectrolytes are ionized and so can interact with each other through ionic bonds most strongly.

However, the negative peak of CD spectra of complexes at 222 nm is shifted to longer wavelengths, 225 nm by $(\text{CH}_2\text{CH}-4-\text{Py})_n$ and 228 nm by $(\text{CH}_2\text{CH}-2-\text{Py})_n$, respectively. Since the Coulombic force seems to be the main force for complexation in this system, the difference of the position of the active sites exerts no influence on the complexation ability but affects the

conformation of the complex like other poly-cations.

The compositions of $(\text{Glu})_m(\text{CH}_2\text{CHPy})_n$ complexes are shown in Table IV. The composition of the complex in base molar ratio is changed from 0.7 to 1.3 in both systems according to a very small change of pH value. This fact is well explained by considering the change of the dissociation states of both component weak polyelectrolytes; *i.e.*, the effective ionic sites for complexation, $-\text{COO}^-$ and $-\text{NH}^+$, are changed sensitively by the pH change of the solution.

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