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Selective Complexation of Macromolecules

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ABSTRACT: It is well known that different macromolecules may interact with each other to form an interpolymer complex. A study is made of the inter-chain macromolecular selective complexation of the type of $P_1 + P_2 + P_3 \rightarrow (P_1\text{–}P_2)$complex $+ P_3$ (where both P_1 and P_3 are able to interact with P_2 individually), taking as an example: P_2 is poly(methacrylic acid) and P_1 and P_3 are chosen at will among poly(4-vinylpyridine), poly(2-vinylpyridine), poly(*N*-vinylpyrrolidone), poly(vinyl alcohol), poly(acrylamide), poly(ethylene oxide), oligo(ethyleneimine), and an integral type polycation. Selective interpolymer complexation is realized through such factors as interaction forces, solvents, ionic strength, chain length, and so on. Moreover, a substitution reaction of the type $(P_2\text{–}P_3)$complex $+ P_1 \rightarrow (P_1\text{–}P_2)$complex $+ P_3$, taking the same samples as mentioned above, progresses through two different paths.

Biological systems are so skillful that a macromolecular chain may effectively select a complementary one to form an interpolymer complex in order to operate very specific functionalities. Synthetic polymers can also form interpolymer complexes,[1–4] but the ability of a synthetic polymer to select only one component polymer as in biological systems has not yet been realized except in the several specific systems of a pair of polymers which include one of a complementary base pair of nucleic acid individually, e.g., poly(A)–poly(U) and poly(I)–poly(C).[5,6] The authors have already reported the complexation mechanisms in various systems composed of synthetic polymers.[7–9] The interpolymer complex formation of synthetic polymers is controlled by many factors, such as interaction forces, solvents, ionic strength, temperature, and so on. These factors suggest that the selective interpolymer complexation can be realized under suitable conditions.

Experimental Section

Materials. Poly(methacrylic acid)(PMAA). Purified methacrylic acid (MAA) (distilled twice in vacuo, bp 63 °C (12 mm Hg)) was polymerized with $K_2S_2O_8$ as an initiator in aqueous solution. The monomer concentration was 0.2 mol/L and the polymerization time was 5 h in nitrogen atmosphere at 50 °C. The reaction mixture was evaporated and dissolved into methanol and then reprecipitated twice from ethyl acetate. The molecular weight of the polymer was calculated from viscosity data,[10] and $\overline{M}_w$ was calculated to be 6.8×10^4.

Poly(L-glutamic acid) (PGA). A 0.5–1.0 wt % aqueous solution of poly(sodium L-glutamate) ($\overline{M}_w = 50\,000$) was passed through a column, 3 cm in diameter and 30 cm in length, filled with an ion-exchange resin, Dow Chemical IR-120, and the pH of the eluent was 3.3. The pH of the solution was then adjusted by partially neutralizing PGA with 0.1 N NaOH aqueous solution.

Poly(4-vinylpyridine) (P4VP) and Poly(2-vinylpyridine) (P2VP). P4VP and P2VP were prepared by the radical polymerization 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 h under nitrogen atmosphere. The monomers and the initiator were purified by distillation or recrystallization according to the usual manner. The resulting polymers were reprecipitated twice from ethyl ether. The degrees of polymerization ($\overline{P}_n$) of the polymers obtained were 49 for P4VP and 108 for P2VP, respectively, by means of vapor-pressure osmometry in methanol.

Poly(*N*-vinylpyrrolidone) (PVPo) and Poly(acrylamide) (PAAm). Purified monomers (bp 94–96 °C (13 mm Hg) for *N*-vinylpyrrolidone and mp 84–85 °C for acrylamide) were polymerized by radical polymerizations in methanol under nitrogen atmosphere at 70 °C for 5 h ([monomer] = 2.0 mol/L, [AIBN] = 0.02 mol/L). The products were diluted with methanol and then reprecipitated from ethyl acetate. The degrees of polymerization of the polymers thus obtained were 96 for PVPo and 82 for PAAm, respectively, by means of vapor-pressure osmometry in methanol.

Poly(vinyl alcohol) (PVOH) and Poly(ethylene oxide) (PEO). PVOH used was a commercial one reprecipitated twice from methanol, having a degree of polymerization about 1000. PEO used were commercial materials with different chain lengths. These samples were reprecipitated twice from ethyl ether, and their molecular weights ($\overline{M}_w$) were given as 1 400, 2 900, 8 000 and 140 000.

Poly(sodium styrenesulfonate) (NaSS), poly(*N,N,N′,N′*-tetramethyl-*N*-*p*-xylyleneethylenediammonium dichloride) (2X), and Oligo(ethyleneimine) (OEI). These polymers were prepared in the same manners as those in a previous paper.[11] The degree of sulfonation of NaSS was 77.1%. OEI used was a pentaethylenehexamine.

Complexation and Measurement. The solutions of three-component polymers (10^{-2}–10^{-1} unit mol/L) were mixed together, and the precipitates were separated from the solutions by means of a centrifuge (12 000 rpm for 1 h) or filtration. The precipitates were dried in vacuo to constant weights, and then they were subjected to elemental analyses, infrared spectroscopy, and weighing. The supernatant solutions were subjected to ultraviolet spectroscopy, circular dichroism, and viscosity measurements (30 ± 0.05 °C). These supernatant solutions were dried and then recovered materials were subjected to the elemental analyses and infrared spectroscopy.

The transmittancy of the complex solutions (one of the parameters of the ability of the complex formation) was measured in the systems of two or three component polymers (2.5×10^{-3}–5×10^{-3} unit mol/L) at room temperature using a Shimadzu spectronic 20-type photoelectric colorimeter at 420 nm.

Results and Discussion

Selective Interpolymer Complexation. Macromolecules can interact with each other through such secondary binding forces as Coulombic force, hydrogen bond, van der Waals

Table I
The Effect of the Dissociation States of the Component Polelectrolytes on the Selective Interpolymer Complexation

Sample A	Sample B	Sample C	Solvent	pH	Init concn, mg A	Init concn, mg B	Init concn, mg C	Yield, mg	Element. Anal. C	Element. Anal. H	Element. Anal. N	Element. Anal. C/N	IR, cm^{-1}	OD_{263}[a]
P2VP	PVPo	PMAA	H_2O–MeOH (1:1)	3.0	52.6	55.6	43.6	99.1	59.1_4	7.6_5	7.0_4	8.4	1660	1.8
P2VP	PVPo	PMAA	H_2O–MeOH (1:1)	6.0	52.6	55.6	43.6	82.2	68.9_1	7.0_1	7.2_8	9.5	1600	≈0
PMAA	PEO	OEI	H_2O	2.0	21.8	11.0	9.5	29.5	55.1_6	7.8_2	0.6_3	88.2		
PMAA	PEO	OEI	H_2O	5.0	21.8	11.0	9.5	25.9	69.3_4	8.5_2	10.3_5	6.7		
PMAA	PEO	OEI	H_2O	9.0	21.8	11.0	9.5	0						

[a] The optical density at 263 nm of the supernatant solution.

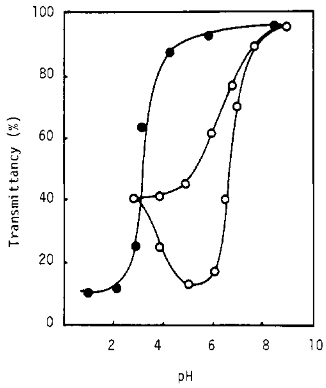


Figure 1. pH Dependence of complexation: (○) OEI–PMAA; (●) PEO–PMAA, [OEI] = [PEO] = [PMAA] = 5.0×10^{-3} unit mol/L, at 25 °C.

force, and hydrophobic interaction. In concentrated systems, interpolymer complexes are formed almost stoichiometrically as precipitates or coacervates which are soluble only in the specific solvents, e.g., ternary solvents, water–acetone–KBr.[12] Even under the same condition, the total free-energy change for complexation varies with the kind of component polymer:

$$\Delta F_{\mathrm{tot}} = \Delta F_{\mathrm{c}} + \Delta F_{\mathrm{hg}} + \Delta F_{\mathrm{hp}} + \ldots \qquad (1)$$

where ΔF_{tot} means the total free-energy change and ΔF_{c}, ΔF_{hg}, and ΔF_{hp} mean the free-energy changes due to Coulombic force, hydrogen bonding, and hydrophobic interaction, respectively. In addition, steric factors, e.g., conformations and/or steric hindrance effects, seem to be very important factors for specific polymer interaction. Since a cooperative interaction is a concequence of the interpolymer complexation, the number of active sites on the polymer chain is an additional factor.

The cooperative inter-chain macromolecular complexation with selectivity is explained as follows

$$\mathrm{P_1 + P_2 + P_3 \rightarrow (P_1\text{–}P_2)complex + P_3} \qquad (2)$$

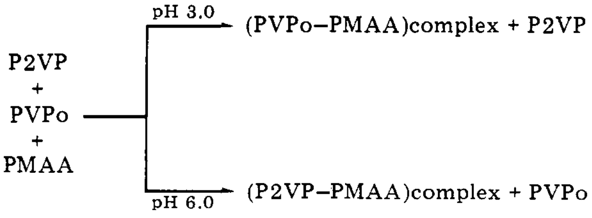
where P_1 and P_3 are able to interact with P_2 individually; that is,

$$\mathrm{P_1 + P_2 \rightarrow (P_1\text{–}P_2)complex} \qquad (3)$$

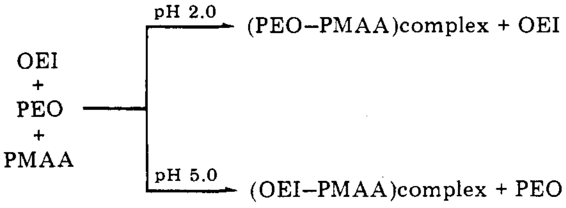
$$\mathrm{P_3 + P_2 \rightarrow (P_3\text{–}P_2)complex} \qquad (4)$$

In this paper, P_2 is poly(methacrylic acid) (PMAA) and P_1 and P_3 are the polymers which can interact with P_2 through various kinds of secondary binding forces: e.g., poly(4-vinylpyridine) (P4VP), poly(2-vinylpyridine) (P2VP), poly(*N*-vinylpyrrolidone) (PVPo), poly(acrylamide) (PAAm), poly(vinyl alcohol) (PVOH), poly(ethylene oxide) (PEO), oligo(ethyleneimine) (OEI), poly(L-glutamic acid) (PGA), poly(sodium styrenesulfonate) (NaSS), and poly(*N*,*N*,*N*′,*N*′-tetramethyl-*N*-*p*-xylyleneethylenediammonium dichloride) (2X).

In Table I, the complexation conditions, the analytical data of the formed complexes, and UV spectra of the supernatant solutions in the systems of P2VP–PVPo–PMAA and OEI–PEO–PMAA are shown. In the P2VP–PVPo–PMAA system, at low pH region, the optical density of P2VP in the supernatant solution at 263 nm is almost the same as that of P2VP in the initial solution (ϵ 2600). From the result of the elemental analysis, it is found that the precipitate is composed of the equimolar component polymers of PMAA and PVPo (calculated value as the equimolar complex of PMAA–PVPo: C, 60.89; H, 7.67; N, 7.10). On the other hand, at high pH region, the optical density of the supernatant solution at 263 nm is very much decreased and more than 85% of P2VP is precipitated with PMAA. The IR spectrum of the precipitate does not show the peak at 1660 cm^{-1} ($\nu_{C=O}$) but 1600 cm^{-1} (ν_{C-N}) of PVPo. The precipitate, however, does not seem to be the equimolar complex of P2VP–PMAA (calculated value as the equimolar complex of P2VP–PMAA: C, 69.08, H, 6.85; N, 7.33), because the dissociation states of both weak polyelectrolytes, PMAA and P2VP, are very dependent on pH of the solution.[13] In the case of the OEI–PEO–PMAA system, from the elemental analysis, it is found that OEI included in the precipitate at low pH region is less than that at high pH region (calculated value as the equimolar complex of OEI–PMAA: C, 55.79; H, 8.59; N, 10.85; C/N, 5.14.) (calculated value as the equimolar complex of PEO–PMAA: C, 55.37; H, 7.75; N, 0; C/N, ∞). Moreover, in the higher pH region, e.g., pH 10, the precipitate is not obtained. From these results, it can be seen that the selective interpolymer complex formations can be represented by the following equations.



(5)



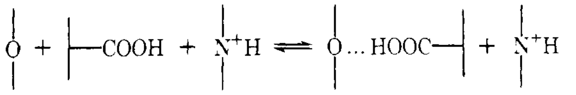
(6)

These systems are composed of a weak polyacid, a weak polybase, and a polymer having an ability to form hydrogen bonding; therefore, the complexation is influenced by pH change of the solution as follows

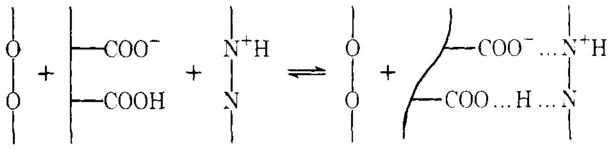
Table II
Selective Interpolymer Complexation through Hydrogen Bonds in Aqueous Solution at pH 2.0

Sample			Init. concn, mg			Yield, mg	Element. Anal.				IR, cm^{-1}
A	B	C	A	B	C		C	H	N	C/N	
PAAm	PVOH	PMAA	35.5	22.0	43.6	76.2	54.3_1	7.8_2	8.7_6	6.5	1670
PAAm	PEO[a]	PMAA	35.5	22.0	43.6	75.1	52.9_5	7.5_8	8.5_4	6.2	1670
PAAm	PEO[b]	PMAA	35.5	22.0	43.6	67.1	56.2_4	7.9_1	0.3_5	163	
PAAm	PVPo	PMAA	35.5	55.6	43.6	78.9	54.6_8	8.0_2	8.8_2	6.2	1670

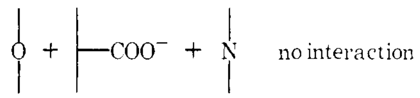
[a] $\overline{M_w} = 2.9 \times 10^3$. [b] $\overline{M_w} = 1.4 \times 10^6$.



$$O + \text{—COOH} + N^{+}H \rightleftharpoons O\cdots HOOC\text{—} + N^{+}H \quad (7)$$



(8)



$$O + \text{—COO}^{-} + N \quad \text{no interaction} \quad (9)$$

That is, (1) in the acidic region, weak polybases (P2VP and OEI) are almost all protonated but a weak polyacid (PMAA) is scarcely dissociated, thus the complex of PMAA with the polymers (PEO and PVPo), which have an ability to form hydrogen bonding with PMAA, is preferentially formed. (2) In the neutral pH region, both a weak polyacid and weak polybases are ionized partially, thus the polyelectrolyte complex is formed. (3) In the alkaline region, a weak polyacid is almost dissociated, while polybases are not protonated, thus neither the polyelectrolyte complex nor the complex through hydrogen bonding are formed. Figure 1 shows the complexation ability of two component polymers of the three against pH change, that is, the decrease of the transmittancy of the solution. PMAA easily makes the complex with PEO in the low pH region but it does not with the OEI in neutral pH region. In addition, in the alkaline region, the complex is not formed. These results coincide well with the observed selective interpolymer complexations in these systems.

In Table II, the complexation conditions, yields, elemental analyses, and so on in the systems of PAAm-PVOH-PMAA, PAAm-PVPo-PMAA, and PAAm-PEO-PMAA at pH 2.0 are shown. In the PAAm-PVOH-PMAA system, the C/N value calculated from the elemental analysis of the precipitate is 6.5 which agrees well with the theoretical value based on assuming an equimolar complex of PAAm-PMAA (Anal. Calcd: C, 53.49; H, 7.06; N, 8.91; C/N, 6.00) and PVOH-PMAA (Anal. Calcd: C, 55.37; H, 7.75; N, 0; C/N, ∞). In the PEO-PAAm-PMAA system, the molecular weight of PEO directly affects the complexation ability. Namely, it appears that PEO with high molecular weight has higher complexation ability with PMAA because of the cooperative reaction of each active site. From these C/N values (theoretical values of C/N are 6.00 for PAAm-PMAA and ∞ for PEO-PMAA, respectively), it is found that PEO ($\overline{M_w}$ = 140 000) forms complex preferentially with PMAA but PEO ($\overline{M_w}$ = 2900) cannot form the complex. On the other hand, in the PAAm-PVPo-PMAA system, PAAm preferentially forms a complex with PMAA which is determined from the C/N value of the precipitate, i.e., the observed value is 6.2 and calculated values as equimolar complexes for PMAA-PVPo and PMAA-PAAm are 8.57 and 6.00, respectively. In these systems, since the complexes are formed mainly through hydrogen bonding, the complexations are always equimolar in unit molar ratio. From these results, it is seen that the selective interpolymer complexations are realized as follows:

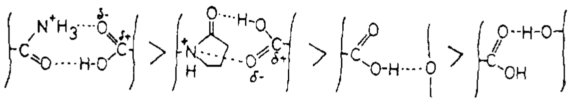
$$\text{PAAm} + \text{PVA} + \text{PMAA} \rightarrow (\text{PAAm–PMAA})\text{complex} + \text{PVA} \quad (10)$$

$$\text{PAAm} + \text{PEO}\ (\overline{M_w} = 1.4 \times 10^6) + \text{PMAA} \rightarrow (\text{PEO–PMAA})\text{complex} + \text{PAAm} \quad (11)$$

$$\text{PAAm} + \text{PEO}\ (\overline{M_w} = 2900) + \text{PMAA} \rightarrow (\text{PAAm–PMAA})\text{complex} + \text{PEO} \quad (12)$$

$$\text{PAAm} + \text{PVPo} + \text{PMAA} \rightarrow (\text{PAAm–PMAA})\text{complex} + \text{PVPo} \quad (13)$$

These results suggest the complexation scheme as follows:



(14)

PAAm and PVPo bind PMAA more strongly than do PEO and PVOH, since PAAm forms complexes with PMAA not only through hydrogen bonding but also through ion–dipole interactions between partially protonated amide groups of PAAm and dipoles of C=O of the carboxylic groups of PMAA. PVPo may also interact through hydrophobic interaction. Kabanov et al. reported[14] selective interpolymer complexation taking as an example PEO + PVOH + PMAA → (PEO–PMAA)complex + PVOH, PVPo + PEO + PMAA → (PVPo–PMAA)complex + PEO, and PEO + PAA + PMAA → (PMAA–PEO)complex + PAA (PAA means poly(acrylic acid)). As a result, the complexation abilities of various polymers with PMAA, mainly through hydrogen bonding, are placed under the experimental conditions applied in order

$$\text{PEO}(\overline{M_w} = 140000) > \text{PAAm} > \text{PVPo} > \text{PEO}(\overline{M_w} = 2900) > \text{PVOH} \quad (15)$$

In Table III, the typical example of the effect of the conformation on the selective interpolymer complexation in the systems of P4VP–P2VP–PMAA and PGA–PEO–PMAA is shown. The elemental analysis data and IR spectra of the precipitates in the systems of P4VP–PMAA and P2VP–PMAA are almost the same (calculated values based on assuming equimolar complexes of P4VP–PMAA and P2VP–PMAA: C, 69.08; H, 6.85; N, 7.33), so the composition of the selective interpolymer complexation is indicated by the ultraviolet spectra of the supernatant solutions. P2VP and P4VP show peaks at 263 nm (ϵ_{263} 2600) and at 256 nm (ϵ_{256} 1700) respectively, and the UV spectrum of the supernatant solution has a peak at 263 nm and its optical density is almost the same as the initial solution of P2VP. In the PGA–PEO–PMAA system, the composition of the supernatant solution is measured by means of circular dichroism (CD) spectrometry, because under the experimental condition applied, PGA holds a helical structure, the reduced molecular ellipticity [θ'] of which is equal to 34 000–40 000. From the result shown in Table III it is found that almost all of PGA exists in the su-

Table III
The Effect of the Conformation on the Selective Interpolymer Complexation

Sample					Init. concn, mg			Yield,	Element. Anal.			λ_{max},[a]	$[\theta']$
A	B	C	Solvent	pH	A	B	C	mg	C	H	N	nm	$\times 10^{-4}$ [a]
P4VP	P2VP	PMAA	H_2O–MeOH (1:1)	5.0	52.6	52.6	43.6	96.8	68.7_4	6.8_9	7.1_8	263	
PGA	PEO	PMAA	H_2O	3.5	75.5	22.0	43.6	65.3	55.1_8	7.9_1	0		2.85

[a] These data were obtained by measuring the λ_{max} and $[\theta']$ of the supernatant solution.

Table IV
Selective Interpolymer Complexation in NaSS–2X–PMAA System

Sample				Init. concn, mg			Yield,	Element. Anal.				
A	B	C	pH	A	B	C	mg	C	H	N	C/N	OD_{265}[a]
NaSS	2X	PMAA	4.0	118.7	72.8	43.6	181.7	64.1_8	9.1_6	4.4_0	14.6	≈0
NaSS	2X	PMAA	9.0	118.7	72.8	43.6	169.6	61.5_0	10.1_4	3.9_9	15.4	≈

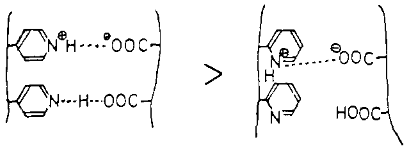
[a] The optical density at 265 nm of the supernatant solution.

pernatant solution. The selective interpolymer complexations in these systems then are,

$$\text{P4VP} + \text{P2VP} + \text{PMAA} \rightarrow \text{(P4VP–PMAA)complex} + \text{P2VP} \quad (16)$$

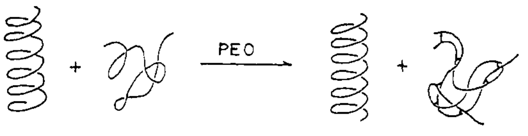
$$\text{PGA} + \text{PEO} + \text{PMAA} \rightarrow \text{(PEO–PMAA)complex} + \text{PGA} \quad (17)$$

Steric hindrance plays an important role on complexation in eq 16. Coulombic force is a longer range attraction, the interaction force being inversely proportional to the square of the distance between two ionic sites. Furthermore, the electrostatic interaction and hydrogen bonding appeared to be coexisting in this system. Hydrogen bonding is a rather short-range interaction with steric factors of importance. So the selective interpolymer complexation takes place as shown in eq 18.



(18)

The difference between α-helical structure and random coil plays an important role on complexation in eq 17. The distance between two carboxylic groups, which are of fixed configuration on the α-helical structure of PGA, does not match the distances of two ether oxygens of PEO. Thus PEO interacts preferentially with the carboxylic groups of a random structure of PMAA.



(19)

In Table IV, the results of a selective interpolymer complexation in the system of strong acid–weak acid–strong base, e.g., NaSS–PMAA–2X, are shown. Within any pH region, two broad peaks at about 260 nm belonging to NaSS (λ_{max} 262 nm, ϵ_{262} 3×10^2) and 2X (λ_{max} 269 nm, ϵ_{269} 4×10^2) are found to disappear with the complexation. Moreover, the C/N value found in the precipitate is about 15, the same value as the calculated value based on assuming the equimolar complex of NaSS–2X (C, 64.38; H, 6.64; N, 4.32; C/N, 14.92). From these results, it is deduced that NaSS and 2X preferentially form an equimolar complex in any pH range.

$$\text{NaSS} + \text{2X} + \text{PMAA} \xrightarrow{\text{at any pH}} \text{(NaSS–2X)complex} + \text{PMAA} \quad (20)$$

At pH 4.0, the carboxylic acid groups of PMAA are principally in the acid form, while the NaSS are almost ionized. Under these conditions, however, NaSS and 2X interact with each other selectively. Even at pH 9.0, where most of the carboxylic groups of PMAA are dissociated, only the NaSS–2X complex is formed. These results suggest that the hydrophobicity of NaSS is larger than that of PMAA so that only NaSS may react with 2X owing to the coexistence of Coulombic force and hydrophobic interaction.

The selective interpolymer complexation based on the difference of the chain length of PEO is shown in Table V. The relative viscosities of the original solutions of PEO with different chain length ($\overline{M_w}$ = 1 400, 8 000, and 140 000) and their mixed solution are 0.072, 0.193, 0.346, and 0.202 at 30 °C, respectively. The relative viscosity of the supernatant solution without complex precipitate was 0.124 which is very similar to the one of the equimolar mixture of PEO ($\overline{M_w}$ = 8000) and PEO ($\overline{M_w}$ = 1400), 0.134. When PMAA was added to the solution, the complex precipitate was obtained. The yield was 63.4 mg and the relative viscosity of the final supernatant solution is 0.078, which was very similar to the one of the solution of PEO ($\overline{M_w}$ = 1400), 0.072.

$$\text{PEO}(\overline{M_w} = 1.4 \times 10^6) + \text{PEO}(\overline{M_w} = 8000) + \text{PEO}(\overline{M_w} = 1400) + \text{PMAA} \rightarrow (\text{PEO}(\overline{M_w} = 1.4 \times 10^6)\text{–PMAA})\text{complex} + \text{PEO}(\overline{M_w} = 8000, 1400) \quad (21)$$

The authors have already found that a cooperative interaction between macromolecules was a very important factor in the interpolymer complexation and that because of this fact there was a critical chain length (a minimum chain length for polymers to form a stable complex) for complexation. A critical chain length in the PMAA–PEO system is about 40 in the degree of polymerization of PEO.[15] Since PEO ($\overline{M_w}$ = 1400, P_n = 32) is below the critical chain length, it can not form a complex with PMAA. However, although both PEO ($\overline{M_w}$ = 140 000) and PEO ($\overline{M_w}$ = 8000) have chain lengths over the critical chain length, only PEO ($\overline{M_w}$ = 140 000) forms a complex with PMAA. From these results, it is clear that the complexation ability of PEO with PMAA gets larger in accordance with the change of the chain length even beyond the

Table V
The Effect of the Chain Length of the Component Polymer on the Selective Interpolymer Complexation in the PMAA–PEO System

Sample				Init. concn, mg				Yield, mg	η_{sp}/c,[a] dl/g
A	B	C	D	A	B	C	D		
PMAA	PEO-I	PEO-II		43.6	22.0	22.0		64.8	0.074
PMAA	PEO-I		PEO-III	43.6	22.0		22.0	65.2	0.076
PMAA		PEO-II	PEO-III	43.6		22.0	22.0	66.7	0.187
PMAA	PEO-I	PEO-II	PEO-III	43.6	22.0	22.0	22.0	67.1	0.124
PMAA		Solution[b]		43.6				64.5	0.086

[a] In H_2O, at 30 (±0.05) °C. [b] Supernatant solution obtained from the PMAA–PEO-I–PEO-II–PEO-III system. The molecular weights of PEO-I, PEO-II, and PEO-III are 1400, 8000, and 1.4×10^6, respectively. pH 2.0.

Table VI
Substitution Reaction of the Interpolymer Complexes in the System of PMAA–PEO–2X

Sample				Init. concn, mg			Yield, mg	Element. Anal.				IR, cm^{-1}	OD_{269}
A	B	C	pH	A	B	C		C	H	N	C/N		
PMAA	PEO	2X	1.8	43.5	22.0	72.8	58.3	54.3_4	7.8_1	0	∞	1750	1.9
PMAA	PEO	2X	5.0	43.5	22.0	72.8	59.6	52.1_4	7.4_3	1.8	28.8		1.4
PMAA	PEO	2X	11.0	43.5	22.0	72.8	98.6	63.8_0	9.4_5	6.5_8	9.7	1400	≈0

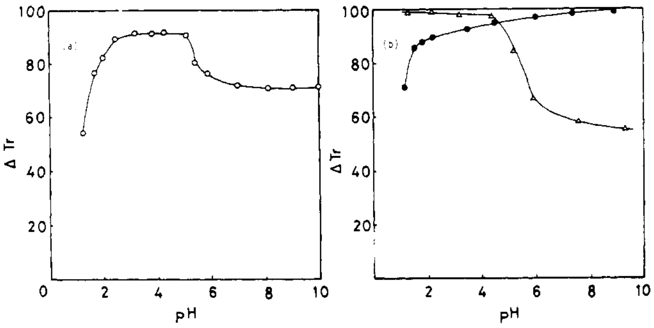


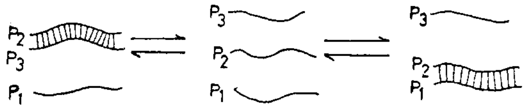
Figure 2. pH dependence of the cooperative inter-chain substitution reaction in the system of 2X–PEO–PMAA: (a) ternary component polymers; (b) two-component polymers of the three, (●) PMAA–PEO, (△) PMAA–2X, [PMAA] = [PEO] = [2X] = 2.5×10^{-3} unit mol/L.

critical chain length because of the cooperative interaction between each active site.

Substitution Reaction. As mentioned above, selective interpolymer complexation is affected by many factors. Especially as shown in Table I, the changes of factors such as pH of the solution can control the selective interpolymer complexation. In these systems, it is expected that the cooperative inter-chain macromolecular substitution reaction of the type

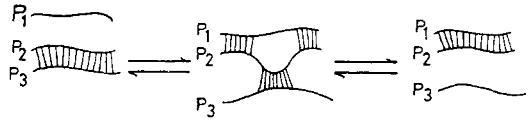
$$(P_2\text{–}P_3)\text{complex} + P_1 \rightarrow (P_1\text{–}P_2)\text{complex} + P_3 \quad (22)$$

could take place. Such substitution reactions may proceed via two mechanisms: an open system and a closed system. (1) An open system



(23)

(2) A closed system

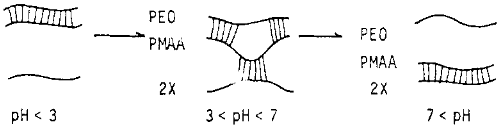


(24)

In an open system, the complex once formed is dissociated into two component polymers according to the changes of environmental factors, and then a different pair of component polymers forms another type of complex. On the contrary, in a closed system, before the complex once formed is completely destroyed, the third component polymer interacts with the complex to form a ternary component polymer complex, and then the first component polymer is dissociated from the ternary complex. In the closed system, the substitution reaction, especially the releasing process of the first component polymer, seems to be accelerated when the interaction force between a parent polymer matrix chain and the third component polymer chain is stronger.

As shown in Table I, in the system of PEO–OEI–PMAA, the substitution reaction proceeds through an open system. This result indicates that the interaction force between PMAA and protonated OEI is so weak that the protonated OEI–PMAA complex cannot be formed until the PEO–PMAA complex is completely destroyed, because it is necessary in order to form a complex of PMAA with PEO that both component weak polyelectrolytes are ionized and because the chain length of OEI is low (a critical chain length for making the polyelectrolyte complex in the PMAA–OEI system is about 4–5[8,13]).

The results of the substitution reaction of the 2X–PEO–PMAA system are shown in Table VI and Figure 2. The PEO–PMAA complex and the 2X–PMAA complex are preferably formed in low or high pH region respectively (see Figure 2b), but in the intermediate pH region the 2X–PEO–PMAA ternary polymer complex is formed except that the precipitate yield is very low (see Figure 2a). Namely, in the 2X–PEO–PMAA system a substitution reaction takes place through a closed system. The decrease of the precipitate yield of the ternary polymer complex is well explained by the scheme as follows



(25)

that is, a part of the nonbonding surplus component polymer chain makes the complex itself hydrophilic, and because of this the ternary polymer complex is very soluble to an aqueous solution.

As a result, a study of such selective interpolymer complexation and substitution reaction would be expected to clarify the fundamental phenomenon of specific polymer reaction in biological systems and the polymer effects of synthetic macromolecules.

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Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. 15. Arginine Parameters from Random Poly(hydroxybutylglutamine-*co*-L-arginine)[1]

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ABSTRACT: Water-soluble copolymers containing L-arginine and N^5-(4-hydroxybutyl)-L-glutamine were prepared by copolymerization of the *N*-carboxy-α-amino acid anhydrides of N^{δ}-*tert*-butyloxycarbonyl-L-ornithine and γ-benzyl L-glutamate, followed by aminolysis with 4-amino-1-butanol, by removal of the *tert*-butyloxycarbonyl protecting group, and by treatment with *O*-methylisourea. The copolymers were fractionated and characterized, and the thermally induced helix-coil transitions of these copolymers were studied in water at neutral pH in the presence and in the absence of KCl. The Zimm–Bragg parameters σ and s for the helix-coil transition in poly(L-arginine) in aqueous solution were deduced from an analysis of the melting curves of the copolymers in the manner described in earlier papers. The computed values of s indicate that L-arginine is a weak helix-making residue at low temperature and a weak helix-breaking residue at high temperature in aqueous solution. The results were found to be in good agreement with those obtained earlier in conformational analyses of arginyl residues in proteins.

The use of the "host–guest" technique for the evaluation of the helix–coil stability constants of various amino acids in water has been illustrated in earlier papers of this series, the latest of which was paper 14.[2] In the present paper, this approach is extended to L-arginine. In the "host–guest" technique, a water-soluble, α-helical host homopolymer with nonionizable side chains is selected, and various amounts of a guest residue are incorporated into it to form random copolymers. The use of random copolymers, in which L-arginine is the minor component, allows the measurement of the helix–coil transition properties of charged arginine residues at neutral pH in the absence of long-range electrostatic interactions between arginine residues.[3] Thus, it is possible[2,3] to determine the Zimm–Bragg[4] parameters σ and s for the guest residues by examining their influence on the helix–coil transition properties of the host homopolymers. L-Arginine residues are incorporated into a copolymer with an N^5-(4-hydroxybutyl)-L-glutamine host, and the thermally induced helix–coil transition in these copolymers in water at neutral pH is examined.

The synthesis of water-soluble random copolymers of L-arginine with N^5-(4-hydroxybutyl)-L-glutamine is described in section I, and the experimental characterization of these copolymers and their melting behavior in aqueous solution are presented in section II. Finally, in section III, the data are analyzed by means of an appropriate form of the theory[5] to determine the helix–coil stability parameters of L-arginine in water. These are compared with empirical observations on the behavior of this residue in proteins.

I. Experimental Section. Preparation and Characterization of the Copolymers

The synthesis of the copolymers was achieved by first copolymerizing the *N*-carboxyanhydrides (NCA's) of N^{δ}-*tert*-butyloxycarbonyl-L-ornithine and γ-benzyl L-glutamate in dioxane using sodium methoxide as an initiator. The resulting copolymers were then converted to the 4-hydroxybutylglutamine derivatives by treatment with 4-amino-1-butanol, and, afterwards, the δ-amino protecting group of L-ornithine was removed using 3 N HCl. The water-soluble copolymers were then treated with *O*-methylisourea in water at pH 10.0 at 0–4 °C to give copolymers of N^5-(4-hydroxybutyl)-L-glutamine with L-arginine [see Katchalski and Spitnik[6] for the preparation of poly(L-arginine) from poly(L-ornithine)].

A. Materials. The solvents and reagents used for the preparation and purification of the starting amino acid derivatives and the NCA's were purified shortly before use (see previous papers in this series[2,3]). Acetonitrile was dried over molecular sieves (4 Å) for 2 days and then refluxed and distilled over calcium hydride. L-Ornithine was purchased from Mann Research Labs., and *tert*-butyloxycarbonyl azide and *O*-methylisourea hydrogen sulfate were purchased from Aldrich Chem. Co.

B. Synthesis. *N*-Carboxyanhydrides. N^{δ}-*tert*-Butyloxycarbonyl-L-ornithine was prepared from the copper complex of L-ornithine and *tert*-butyloxycarbonyl azide, followed by treatment of the isolated Cu complex with hydrogen sulfide, according to Tesser and Schwyzer.[7]

To a suspension of N^{δ}-*tert*-butyloxycarbonyl-L-ornithine and AgCN in freshly distilled acetonitrile, a solution of phosgene in di-