A New Class of Biodegradable Thermosensitive Polymers. 2. Hydrolytic Properties and Salt Effect on the Lower Critical Solution Temperature of Poly(organophosphazenes) with Methoxypoly(ethylene glycol) and Amino Acid Esters as Side Groups

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ABSTRACT: The hydrolytic properties of the novel biodegradable thermosensitive poly(organophosphazenes) with methoxypoly(ethylene glycol) (MPEG) and amino acid esters as side groups have been studied by means of gel permeation chromatography and ^{31}P and ^{1}H NMR spectroscopy and by identification of the hydrolysis products. The polymers substituted with α-amino acid esters were hydrolyzed faster than that with *β*-amino acid ester. The higher content of the amino acid ester in the polymer backbone caused enhanced hydrolysis. The rate of the polymer degradation decreased in the order of methyl > ethyl > benzyl esters. The polymer hydrolysis occurred more rapidly in both acidic and basic buffer solutions than in the neutral solution. The ^{31}P NMR spectra of the polymers with high content of glycine ethyl ester showed that the polyphosphazene backbone underwent fragmentation mostly to small molecules after incubation in the buffer solution of pH 10 for 26 days. Phosphates and ammonia were formed as hydrolysis products in most cases. The hydrolytic behaviors of the present thermosensitive polyphosphazenes are consistent with the conventional acid-catalyzed degradation mechanism, and a detailed pathway to their hydrolytic degradation is proposed. The salt and pH effects on the thermosensitivity of the polymers were also examined by measuring their lower critical solution temperature (LCST) in aqueous solutions containing various inorganic and organic salts. When various inorganic salts were added to aqueous solutions of the polymers, their salting-in and salting-out effects were found to be mainly dependent on the anions of the salts. On the other hand, in the case of tetraalkylammonium halides which are organic salts, cations seem to play an important role: the salting-in effect is stronger with increasing alkyl chain of the ammonium salt. The aqueous solutions of the polymers showed higher LCST in the acidic solution than in the neutral and basic buffer solutions.

Introduction

Thermosensitive polymers exhibit a reversible phase transition behavior depending on the polymer solution temperature. Such a unique property offers a potential for applications of thermosensitive polymers to drug delivery systems (DDS). However, their application to DDS has been difficult because most of the thermosensitive polymers are nonbiodegradable and some of them are toxic. Biodegradability of polymeric materials is one of the most important factors for their application to a polymer-based drug delivery system. A number of biodegradable polymers have been studied for application to the controlled drug delivery system. Lactide and glycolide copolymers are representative biodegradable polymers, and they have been used for delivery of a wide range of bioactive agents.[1] However, it has been noted that these polymers are degraded in an uncontrolled rate and release acid moieties, which can be problematic for protein drug delivery.[2,3] The degradation rate and pattern of the biodegradable polymers are very important, and they must produce harmless moieties when degraded.

The characteristics of the aqueous solution of a substance reflect mainly the interaction between the solute and water molecules through hydrogen bonding. As for synthetic macromolecules, the configuration of hydrophilic polymers such as poly(vinyl alcohol), poly(vinylpyrrolidone), and poly(ethylene oxide) in water are rather insensitive to addition of water-soluble additives such as urea, but the configuration of macromolecules composed of both hydrophilic and hydrophobic groups is markedly affected by addition of various organic and inorganic salts, and in fact, some of the thermosensitive polymers are readily dissolved in aqueous salt media.[4] Prediction of the LCST of nonionic macromolecules is very important for biological applications such as drug delivery systems, enzyme and cell immobilization, solute separation, immunodiagonostic assays, and purification and partitioning in biotechnology.[5−7] The relative effects of various salts in stabilizing or destabilizing representative thermosensitive polymers such as homopolymer or copolymer of ethylene oxide and *N*-isopropylacrylamide in aqueous solutions are well-known.[8−10] However, for polyphosphazenes, the salt and pH effects on thermosensitivity have not been extensively studied.

Poly(organophosphazenes) have a potential as polymeric carriers for biologically active agents. The advantages of these polymers come mainly from a wide variety of properties that can be designed and attainable by nucleophilic substitution of poly(dichlorophosphazene) with various organic groups. Polyphosphazenes with amino acid ester substituents have been described by Allcock et al.,[11,12] and they are potentially biocompatible and biodegradable to harmless products. Therefore, these polymers have been studied for the preparation of macromolecular prodrugs as well as for the produc-

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Table 1. Characteristics of Poly(organophosphazenes)

polymer	formula	LCST(°C)	M_w ($\times 10^{-4}$)	$t_{1/2}$(days) pH = 5	pH = 7.4	pH = 10	ΔLCST(°C) ($T_{1.0M} - T_{0M}$)[a]
1	$[NP(MPEG350)_{1.42}(GlyEt)_{0.58}]_n$	93.2	4.73	28	>40	>40	−27.0
2	$[NP(MPEG350)_{0.93}(GlyEt)_{1.07}]_n$	77.0	2.24	24	>40	20	
3	$[NP(MPEG350)_{0.99}(GlyEt)_{1.01}]_n$	77.5	3.48				−19.2
4	$[NP(MPEG350)_{0.58}(GlyEt)_{1.42}]_n$	64.5	1.77	14	37	8	−12.0
5	$[NP(MPEG350)_{1.03}(GlyMe)_{0.97}]_n$	88.5	3.08	7	16	5	−25.7
6	$[NP(MPEG350)_{1.00}(GlyBz)_{1.00}]_n$	49.5	2.13	>40	>40	>40	−13.7
7	$[NP(MPEG350)_{1.03}(\beta\text{-}AlaEt)_{0.97}]_n$	70.3	2.18	>40	>40	>40	−18.5
8	$[NP(MPEG350)_{1.00}(AlaEt)_{1.00}]_n$	67.0	3.58	12	>40	12	−19.0
9	$[NP(MPEG350)_{0.97}(MalEt_2)_{1.03}]_n$	65.5	2.24	15	31	26	−25.0
10	$[NP(MPEG350)_{1.01}(AspEt_2)_{0.99}]_n$	60.2	4.40	11	26	13	−16.5
11	$[NP(MPEG350)_{0.80}(HA)_{1.20}]_n$	59.0	2.11	>40	>40	>40	
12	$[NP(MPEG350)_{2.00}]_n$		4.26	>40	>40	>40	

[a] Changes of LCST by addition of NaCl (1.0 M).

tion of drug-loaded microspheres.[13] Several kinds of the thermosensitive poly(organophosphazenes) bearing alkyl ether side groups have been studied as an electrolyte material, but these polymers are nondegradable.[14,15] Recently, we have reported synthesis and characterization of poly(organophosphazenes) with side groups of methoxypoly(ethylene glycol) (MPEG) and amino acid esters, which constitute a new class of biodegradable and thermosensitive polymers.[16] In this paper, we present the results of our study on hydrolytic properties and salts effect on the LCST of these polymers.

Experimental Section

Materials. The ethyl ester of β-alanine (Acros) was prepared by the literature method.[17] Hexylamine (Aldrich) was dried azeotropically with benzene, followed by vacuum-drying, and then stored over molecular sieve 4A. Acetic acid and anhydrous sodium carbonate (Junsei), anhydrous sodium acetate (Kanto), tris(hydroxymethyl)aminomethane (Aldrich), and anhydrous sodium bicarbonate (Shinyo) were used as received in the preparation of buffer solutions. Guaranteed reagent grade LiCl, KCl, NaCl, NaBr, KF, NaI, NH_4I, NH_4Cl, and NH_4Br from commercial sources were used as received. Also, guaranteed reagent grade Me_4NBr, Et_4NCl, Et_4NBr, Et_4NI, n-Pr_4NBr, and n-Bu_4NBr, commercially purchased, were used without further purification.

Polymers. Various thermosensitive poly(organophosphazenes) with MPEG and amino acid esters as side groups were employed in this study. Polymers **1**, **3**−**6**, and **8**−**10** (see Table 1) were prepared in the previous work,[16] and other polymers were prepared by the same procedure using the corresponding substituent reagents and mole ratios. The characteristics of the polymers are listed in Table 1.

$[NP(MPEG350)_{0.93}(GlyEt)_{1.07}]_n$ (2). MPEG350 (17.3 mmol) and glycine ethyl ester (34.5 mmol) were used. Yield: 71%. ^{31}P NMR (acetone-d_6), δ (ppm): 23.58. ^1H NMR(D_2O), δ (ppm): 1.2−1.4 (m, 3H), 2.6−2.7 (m, 2H), 3.2−3.3 (m, 2H), 3.4 (s, 3H), 3.6−3.9 (b, 26H), 4.0−4.4 (b, 4H). Elemental analysis (%) calcd: C, 45.73; H, 7.87; N, 6.06; P, 6.47. Found: C, 44.30; H, 7.73; N, 6.12; P, 6.41.

$[NP(MPEG350)_{1.03}(\beta\text{-}AlaEt)_{0.97}]_n$ (7). MPEG350 (17.3 mmol) and β-alanine ethyl ester (34.5 mmol) were used. Yield: 48%. ^{31}P NMR (acetone-d_6), δ (ppm): 24.01. ^1H NMR (D_2O), δ (ppm): 1.2−1.4 (m, 3H), 2.6−2.7 (m, 2H), 3.2−3.3 (m, 2H), 3.4 (s, 3H), 3.6−3.9 (b, 26H), 4.0−4.4 (b, 4H). Elemental analysis (%) calcd: C, 46.84; H, 8.11; N, 5.36; P, 6.07. Found: C, 47.06; H, 8.23; N, 5.42; P, 6.11.

$[NP(MPEG350)_{0.80}(HA)_{1.20}]_n$ (11). MPEG350 (17.3 mmol) and hexylamine (34.5 mmol) were used. Yield: 68%. ^{31}P NMR (acetone-d_6), δ (ppm): 25.28. ^1H NMR(D_2O), δ (ppm): 0.8−0.9 (m, 3H), 1.2−1.4 (b, 6H), 1.5−1.6 (b, 2H), 2.9−3.2 (b, 2H), 3.4 (s, 3H), 3.6−3.9 (b, 26H), 3.9−4.1 (b, 2H). Elemental analysis (%) calcd: C, 51.41; H, 9.64; N, 7.03; P, 7.27. Found: C, 51.58; H, 9.77; N, 7.09; P, 7.19.

$[NP(MPEG350)_2]_n$ (12). The homopolymer bearing MPEG-350 was prepared by the literature method.[14]

Instruments. Elemental analysis was carried out with Fisons 1108 CHNS Microanalizer and Polyscan 61E ICP. ^1H NMR measurements were made with a Varian Gemini-300 spectrometer operating at 300 MHz in the Fourier transform mode. Proton-decoupled ^{31}P NMR spectra were measured with the same spectrometer operating at 121.4 MHz, using triphenyl phosphate as an external standard. Gel-permeation chromatography was performed to measure the weight-average molecular weight (M_w) of the polymers, using a Waters Associates HPLC/GPC 150C unit and two Ultrahydrogel columns (Ultrahydrogel Linear and 250) connected in line at a flow rate of 0.8 mL/min at 35 °C. Poly(ethylene oxides) (M_w: 600, 900, 1470, 7100, 12 600, 23 000, 46 000, 95 000) were used as standards to calibrate the column.

Measurement of LCST. The phase transition of the polymer solutions (5 wt %) containing different kinds and concentrations of salts (0−1.0 M) was detected visually in a closed glass tube immersed in an oil bath. The LCST was identified as the temperature at which the solution became turbid. The pH dependence of LCST was examined in acetic acid/sodium acetate buffer solutions at pH 2.3, 3.2, 4.2, 5.0, 6.0, 7.0, and 8.0.

In Vitro Hydrolytic Degradation of Poly(organophosphazenes). Time-dependent degradation of the thermosensitive poly(organophosphazenes) was examined in different pH buffer solutions at different temperatures. The poly(organophosphazenes) (74 mg) were dissolved in 2 mL of 0.5 M buffer solutions (acetate buffer of pH 5, tris buffer of pH 7.4, and carbonate buffer of pH 10), which were incubated in water bath at different temperatures (5, 37, and 50 °C). Time-dependent hydrolytic behavior of the polymers was determined in terms of molecular weight decrease of the polymers by GPC. For more detailed hydrolytic study, a large-scale hydrolytic experiment was carried out: After the polymers (518 mg) dissolved in the same buffer solutions (14 mL) were incubated in a water bath at different temperatures for 26 days, the solutions were subjected to GPC analysis and titration for ammonia, phosphate, and alcohol formed. After freeze-drying the polymer solutions, the products were characterized by ^{31}P NMR spectroscopy. The freeze-dried products were also dialyzed extensively with distilled water to remove small molecules, and then the solutions were freeze-dried again. The remaining products were characterized by ^1H NMR spectroscopy and LCST measurement. All the experiments were carried out in duplicate.

Identification of the Hydrolysis Products. The hydrolysis products of the polymers after incubation in aqueous solution were qualitatively examined using the literature method.[12] Phosphates were detected with silver nitrate ($AgNO_3$): $AgNO_3$ (1.0 mL of 1.0 M solution) was added to aliquots taken from the aqueous media, and formation of the yellow precipitate of silver phosphate indicated the presence of phosphate. Ammonia was detected by the ninhydrin test: A n-butanol solution of ninhydrin (0.2%, 1 mL) was added to the aliquots,

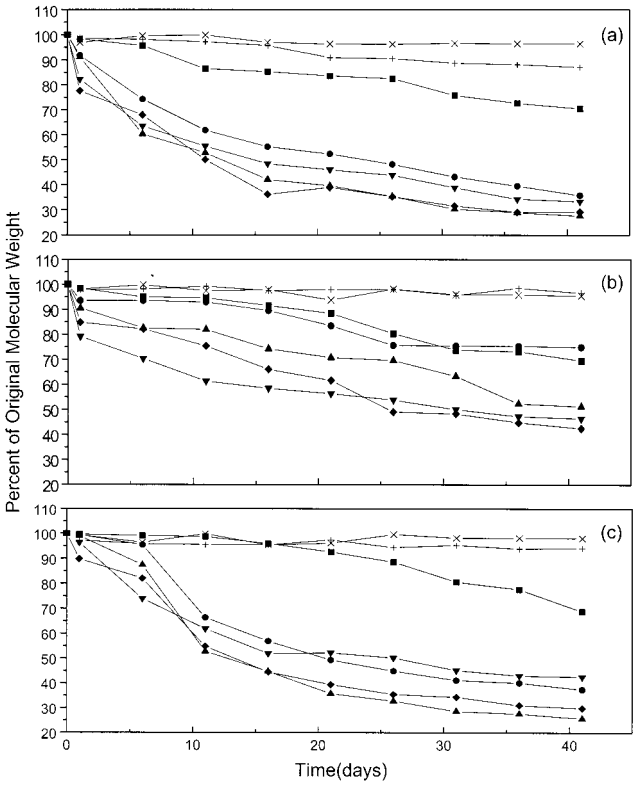


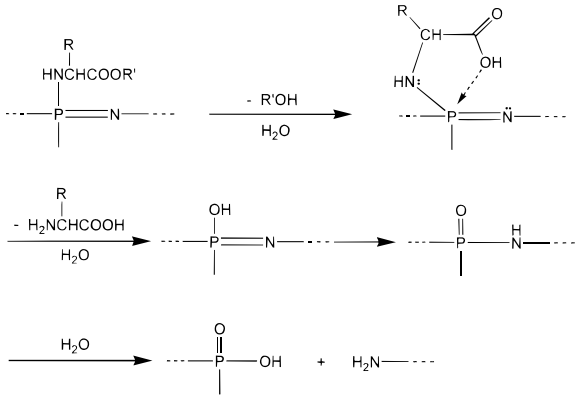
Figure 1. Time-dependent hydrolytic degradation of polymer **2** (●), polymer **7** (■), polymer **8** (▲), polymer **9** (▼), polymer **10** (◆), polymer **11** (×), and polymer **12** (+) at pH 5 (a), pH 7.4 (b), and pH 10 (c) at 37 °C.

and an intensive color appeared within minutes after addition when ammonia was present. Ethanol formed by hydrolysis was identified by ^{1}H NMR spectroscopy (300 MHz): For ethyl alcohol, an aliquot from the aqueous media was heated to 90 °C to distill volatile species into a small size of Dean−Stark trap, and then the liquid collected was subjected to ^{1}H NMR spectroscopy.

Results and Discussion

Hydrolytic Behavior. To study hydrolytic properties of the present thermosensitive polymers, the polymers were dissolved in the buffer solutions of different pH, and their hydrolysis was monitored in terms of molecular weight change by GPC. Figure 1 shows the profiles of the time-dependent molecular weight decrease of the polymers bearing different amino acids in different buffer solutions at 37 °C. The hydrolytic behavior of these poly(organophosphazenes) is dependent on the nature of the substituents and pH of the buffer solutions. The polymers with MPEG and amino acid esters (polymer **1**−**10**) showed a significant molecular weight decline, and their rate of hydrolysis was affected by the structure of the amino acids. Polymer **7** with β-alanine ethyl ester as a side group showed a significantly slower hydrolysis in both the acidic and basic buffer solutions than other polymers carrying α-amino acid esters. This result seems to be due to the structural difference between the α- and β-amino acid esters as side groups, which will be further discussed later in Scheme 1. However, the polymers with MPEG and α-amino acid esters did not show significant differences in hydrolysis. All the polymers with MPEG and amino acid ester pendants underwent hydrolysis faster in both the acidic and basic solutions than in the neutral solution. The difference between the rates of hydrolysis in the acidic and basic solutions was not significant. On the other hand, the poly(organophosphazene) bearing MPEG and hexylamine pendants (polymer **11**) and the homopolymer carrying the MPEG pendant only (polymer **12**) did not show significant molecular weight decrease in all the buffer solutions. Such results give rise to a mechanistic evidence of hydrolysis of the poly(organophosphazenes) bearing amino acid esters as side groups, which is in accord with the previous reports.[12,18,19]

Scheme 1. A Proposed Hydrolytic Pathway of Poly(organophosphazene) with MPEG and Amino Aicd Esters as Side Groups



The hydrolytic degradation of poly(organophophazenes) substituted with amino acid esters has been explained in terms of carboxylic acid-catalyzed degradation.[12,18,19] It has been proposed that the initiation step of hydrolytic degradation of the poly(organophosphazenes) substituted with amino acid esters is hydrolysis of the pendent ester group generating the corresponding free carboxylic acid. The carboxylic acid group generated attacks the polymer backbone, resulting in backbone cleavage. In this mechanism, two important factors, that is, hydrolysis sensitivity of esters and the number of the free carboxylic acid groups formed, should be emphasized. Now the aforementioned observations that the polymers with MPEG and amino acid esters were hydrolyzed faster in both the acidic and basic solutions than in the neutral solution and that the difference between the hydrolysis rates in the acidic and basic solutions was not significant may be explained by the above acid-catalyzed degradation mechanism. The rate of the ester group hydrolysis in aqueous solution is known to decrease in the order of base > acid > neutral solution, and therefore, the ester group may be rapidly hydrolyzed in the basic solution. However, the number of the free carboxylic acid moieties formed in the basic buffer solution is probably not as much as that of the hydrolyzed carboxylate groups, since some of the hydrolyzed carboxylate groups must be in the form of carboxylate anion. Consequently, the difference between the hydrolysis rates in the acidic and basic solutions seems to be insignificant. The observation that both polymers **11** and **12,** which do not have an amino acid ester, were very sluggish in hydrolytic degradation in all the buffer solutions is also consistent with the acid-catalyzed degradation mechanism.

The rate of degradation dependent on the mole ratio of the substituents and ester groups of the amino acids attached to the polymers was studied, and the results are shown in Figure 2. Degradation of the polymers was clearly influenced by the contents of each substituent, and in particular, the high content of glycine ethyl ester

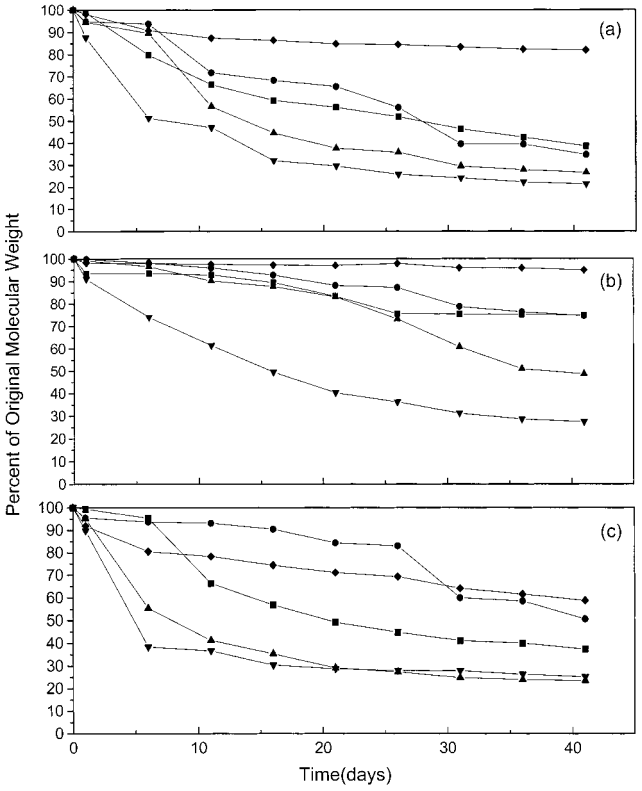


Figure 2. Time-dependent hydrolytic degradation of polymer **1** (●), polymer **2** (■), polymer **4** (▲), polymer **5** (▼), and polymer **6** (◆) at pH 5 (a), pH 7.4 (b), and pH 10 (c) at 37 °C.

groups in the polymer accelerated the polymer degradation. The increase in the content of glycine ethyl ester caused more rapid hydrolysis in the basic and acidic solutions than in the neutral solution. However, polymers **1** and **2** did not show any significant difference in degradation rate in both acidic and neutral solutions although the content of the ester group in polymer **2** is higher than that of polymer **1**. This result seems to be due to the hydrophobicity of the polymers which increases with increasing content of the glycine ethyl ester pendant. Allcock and co-worker have reported that the polyphosphazene substituted with hydrophobic and bulkier phenyalanine ethyl ester was more stable in hydrolysis than the one with glycine ethyl ester.[20] Similarly, Vandrope and co-workers have studied the effect of the hydrophilic poly(ethylene oxide) (PEO) chain length of the polyphosphazene substituted with PEO and glycine ethyl ester on the polymer degradation, and they found that the longer PEO chain increased the rate of the polymer degradation.[20]

The structure of the ester groups significantly affected the polymer degradation, and the order of increasing sensitivity to degradation was in the order of methyl > ethyl > benzyl esters in all the buffer solutions. This result may be explained in terms of the hydrolytic stability and hydrophobicity of the esters.[12] It is known that methyl ester is more easily hydrolyzed in aqueous solution than ethyl and benzyl esters. The difference between the hydrolysis rates of the ethyl and benzyl esters appears to arise from their differences in hydrophobicity and bulkiness of the ester parts.

Finally, the effect of solution temperature on the degradation for polymer **2** is shown in Figure 3. Elevation of the hydrolysis temperature greatly accelerated the degradation process as reflected by the rapid molecular weight decrease of polymer **2** at 50 °C, whereas the same polymer was rather resistant to hydrolysis at 5 °C.

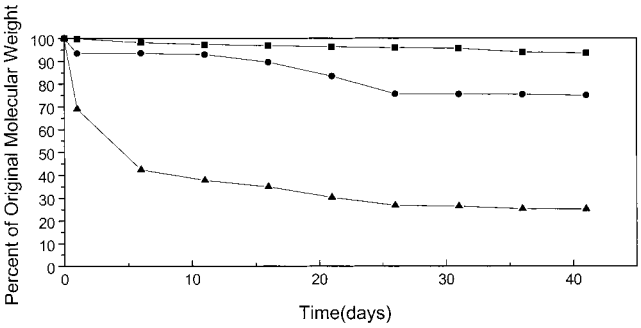


Figure 3. Time-dependent hydrolytic degradation of polymer **2** in the buffer solution of pH 7.4 at 5(■), 37(●), and 50 °C(▲).

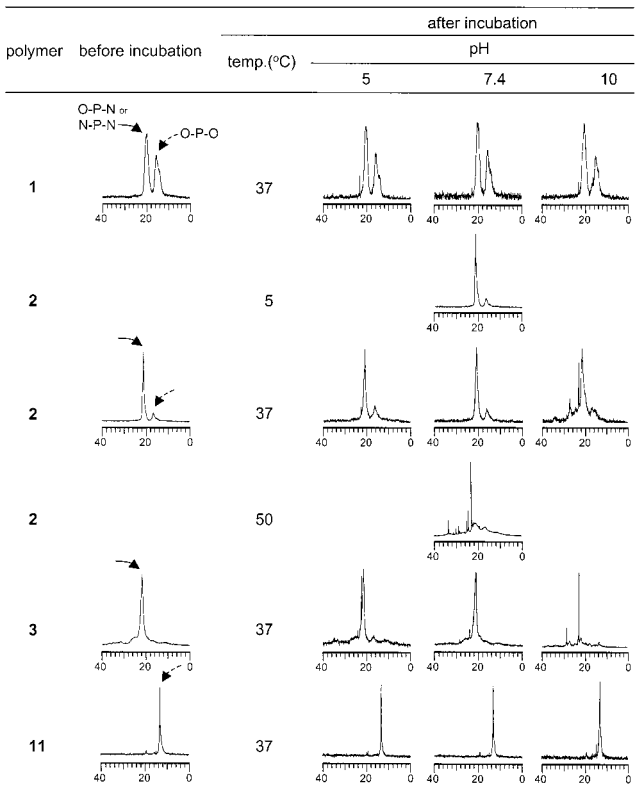


Figure 4. ^{31}P NMR spectra of polymers before and after incubation for 26 days.

Hydrolyzed Products. Characterization of the hydrolysis products of the polymers was carried out by means of ^{1}H and ^{31}P NMR, GPC, and titration methods. The ^{31}P NMR spectra of the poly(organophosphazenes) bearing MPEG and glycine ethyl ester pendants were measured before and after incubation in buffer solutions at different pHs and temperatures for 26 days, and the results are represented in Figure 4. The ^{31}P NMR spectra of polymer **1** with a low content of glycine ethyl ester remained virtually unchanged even after incubation in all the buffer solutions, which indicates that fragmentation of the polyphosphazene backbone into small molecules did not occur to any meaningful extent until the midstage of degradation. However, significant spectral changes were observed for the polymers with increased content of glycine ethyl ester, which was more prominent in the buffer solution of pH 10. The ^{31}P NMR spectra of polymers **2** and **4** after incubation at pH 10 were quite different from those of the polymers before

Table 2. Characteristics of Poly(organophosphazenes) after Incubation for 26 days at 37 °C

								relative mole ratios	
polymer	LCST (°C)	pH	*P* (%)[a]	LCST (°C)[b]	ethanol[c]	phosphate[c]	ammonia[c]	MPEG	$CO_2CH_2CH_3$
1	93.2	5	57	88.5		++	+	1.36	0.64
		7.4	83	97.0		+		1.51	0.49
		10	81		+	+++	++	1.89	0.11
2	77.0	5	55	91.0	++	++	++	1.17	0.83
		7.4	76	89.0	++	+		1.16	0.84
		10	47		++	+++	+++	1.82	0.18
4	64.5	5	41	90.0	+	++	++	1.09	0.91
		7.4	77	96.5	++	+	+	1.19	0.81
		10	33		+++	+++	+++	2.00	0
12		5	83			+			
		7.4	96						
		10	86			+			

[a] The proportion of the molecular weight decrease of the polymers after incubation. [b] LCST after incubation. [c] The relative amount detected.

incubation. Especially, the spectrum of polymer **4** after incubation in the basic solution at 37 °C is entirely different from that of the original, which indicates that the polymer backbone was mostly fragmented to some phosphate moieties. This result is consistent with the GPC data, which show the molecular weight of polymer **4** decreased to almost 33% of the original one when the polymer was incubated in the basic solution for 26 days, as shown in Figure 2 and Table 2. On the other hand, the ^{31}P NMR spectra of the homopolymer with MPEG (polymer **12**) after incubation in all the buffer solutions were almost the same as that of the original one, which is also consistent with the GPC data in Figure 1. The ^{31}P NMR spectra of polymer **2** after incubation in the neutral solution at different solution temperatures also show the same results as the above-mentioned GPC data: The ^{31}P NMR spectrum of polymer **2** after incubation at 50 °C shows a drastic change, implying a remarkable fragmentation of the polymer backbone, whereas the spectra of polymer **2** after incubation at 5 and 37 °C show no significant change from that of the original.

To examine the hydrolysis rate of the ester groups, the ethyl ester group of polymer **2** was monitored before and after incubation by ^{1}H NMR spectroscopy, which showed that hydrolysis of the ethyl ester group was remarkably dependent on the incubation conditions. In the basic solution and at high temperature, rapid hydrolysis of the ester group was observed. For instance, the mole fraction of the ethyl ester group of polymer **2** decreased from 0.54 to 0.09 when polymer **2** was incubated in the buffer solution of pH 10, whereas the fraction was decreased to only 0.42 in the buffer solutions of pH 5 and 7.4. A similar trend was observed for polymers **1** and **4**. The ethanol converted from ethyl esters was detected for polymers **2** and **4**, and its amount was consistently larger in the buffer solution of pH 10 than in the buffer solutions of pH 5 and 7.4. The results of the phosphate titration has shown that phosphate was formed during hydrolysis of almost all the polymers, and its amount increased with increasing extent of the polymer degradation. A similar trend was observed in ammonia titration. However, ammonia was not detected after incubation at pH 7.4, which is likely due to low sensitivity of the titration method. In the case of polymer **12**, only a small amount of phosphate was detected.

Hydrolysis Mechanism. The aforementioned hydrolytic behaviors of the present thermosensitive polyphosphazenes are generally consistent with the carboxylic acid-catalyzed degradation mechanism previously proposed,[12,18,19] and a pathway to the hydrolytic degradation of our thermosensitive polymers is proposed in Scheme 1.

The initiation step is hydrolysis of the pendent ester group generating the free carboxylic acid. The hydroxy group of the carboxylic acid coordinates to the phosphorus atom forming a cyclic structure, and then the phosphorus atom is attacked by a water molecule to release the amino acid with formation of hydroxyphosphazene, which is finally subjected to backbone cleavage. In this hydrolytic mechanism, the mode of attack by the free carboxylic acid to the phosphorus atom can be figured out as shown in Scheme 1. When the free carboxylic acid attacks the phosphorus atom, a five-memberd ring can be formed in the case of the α-amino acid whereas a six-membered ring is formed in the case of the *β*-amino acid. The difference between the rates of formation of the five-membered and six-membered rings has been mentioned in the literature:[21] The rate of formation of the five-membered ring is almost 10^2 times faster than that of the six-membered. From this reason, the free carboxylic acid is assumed to attack the phosphorus atom bonded to the amine group of the amino acid itself rather than the neighboring phosphorus atom. This assumption is supported by the result shown in Figure 1: The hydrolysis rate of the polymers with MPEG and α-amino acid esters was faster than that of the polymer with MPEG and *β*-amino acid esters when their compositions were similar.

Salt Effect on LCST of the Polymers. The homopolymers and copolymers of poly(ethylene oxide) (PEO) are typical thermosensitive polymers, and their thermosensitivity is derived from the interaction through hydrogen bonding between PEO and water molecules.[22] It is accepted that the salt effect on the LCST of the thermosensitive PEO polymers is due to change of water structure by salts. Some salts increase the LCST of the PEO polymers, which is called the "salting-in" effect, while other salts decrease it, which is called the "salting-out" effect. To study the salt effect on the LCST of the present thermosensitive poly(organophosphazenes) carrying MPEG and amino acid ester pendants, various kinds of salts (0−1.0 M) were added to the aqueous polymer solutions (5 wt %), and their LCSTs were measured. The LCST of the aqueous solutions of polymer **3** and polymer **10** changed remarkably by addition of inorganic salts as shown in Figure 5. Most of the inorganic salts except for NaI have shown the salting-out (water structure making) effect on the solution of

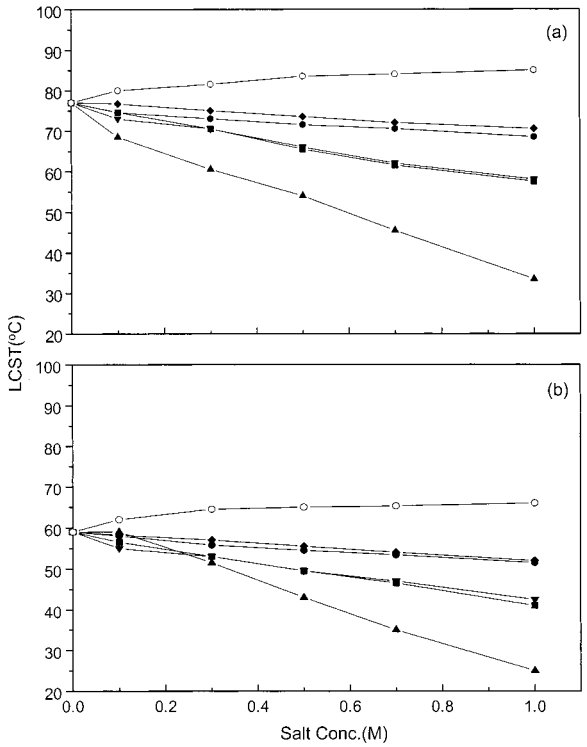


Figure 5. Change of LCST of polymer **3** (a) and polymer **10** (b) solutions by addition of inorganic salts: LiCl (●), KCl (■), KF (▲), NaCl (▼), NaBr (◆), NaI (○).

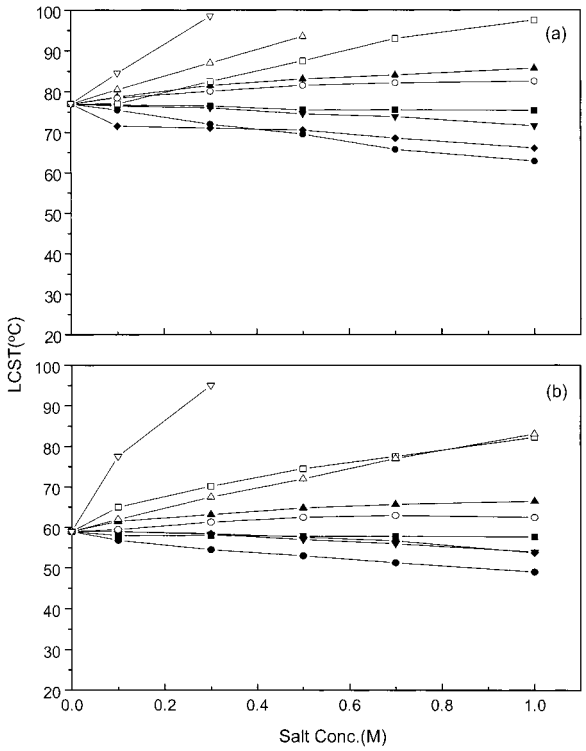


Figure 6. Change of LCST of polymer **3** (a) and polymer **10** (b) solutions by addition of organic salts: NH_4Cl (●), NH_4Br (■), NH_4I (▲), Me_4NBr (▼), Et_4NCl (◆), Et_4NBr (○), Et_4NI (□), Pr_4NBr (△), Bu_4NBr (▽).

the polymer substituted with MPEG and glycine ethyl ester (polymer **3**), which was dependent on both cations and anions. The order of effectiveness of salting out is $K^+ = Na^+ > Li^+$ and $Cl^- > Br^-$. Interestingly enough, I^- appears to give the salting-in (water structure breaking) effect. These results are consistent with the studies on the poly(vinylmethyloxazolidinone) solution by Hippel and co-workers.[23] They have noted that cations and anions are effective in altering the cloud point of the polymer solution in the order of salting-in to salting-out effect: $Li^+ > NH_4^+ > K^+$, Na^+ and $ClO_4^- > Br^- > Cl^- > F^- > CO_3^{2-} > SO_4^{2-}$. Similarly, Horne and co-workers[24] have reported the salt effect on the cloud point of the PEO system, and the order of the salting-in to salting-out effect of cations and anions was almost the same as the above results. It is generally accepted that the anion is important in the salt effect. In particular, a large ion such as I^- is classified as a water structure breaker while small and/or highly charged electrostrictive ions such as F^- and Cl^- are water structure makers, and as such, the small fluoride anion shows a strong salting-out effect. In the case of polymer **3**, increasing the concentration of NaI, a water structure breaker, seems to increase hydrogen bonding between molecules of water and the polymer, resulting in increase of its LCST. On the contrary, other inorganic salts, water structure makers, decrease the interaction of water with polymers, resulting in decrease of LCST. A similar trend was observed in the polymer substituted with MPEG and L-aspartic acid ethyl ester (polymer **10**). The salting-in to salting-out effect of cations and anions of inorganic salts on the polymer **10** solution was almost the same as that of the polymer **3** solution, which indicates that the change of the LCST of the polymers seems to be more susceptible to the hydrophilic MPEG than the hydrophobic amino acid ethyl esters of the polymers.

Figure 6 shows the effect of organic salts on the LCST of the solutions of polymer **3** and polymer **10**. The effect of organic salts was also dependent on both cations and anions. As far as the effect of the R_4N^+ cation is concerned, the larger R_4N^+ ion produced the greater salting-in effect following the order of $Bu_4N^+ > Pr_4N^+ > Et_4N^+ > NH_4^+$, whereas Me_4N^+ showed slightly a salting-out effect. It is known that large R_4N^+ ions enhance the hydrogen-bonded structure because their very hydrophobic nature induces binding of these cations to the hydrophobic part of the polymer, resulting in increase in the overall hydrophilicity of the polymer molecules.[25] The fact that salting-in effect of Bu_4N^+ is larger in polymer **10** than in polymer **3** is likely due to the difference between their hydrophobicity. For the ammonium and tetraethylammonium halides, halide anions give a large effect on the LCST of the polymers in the order of salting-in to salting-out: $Et_4NI > NH_4I > Et_4NBr > NH_4Br > Et_4NCl > NH_4Cl$. These results are consistent with the results presented earlier in Figure 5.

Table 1 lists the NaCl effect on the LCST of the polymers. The change of the LCST of the polymer solutions by NaCl was dependent on the mole ratio of the substituents, and the LCST decreased more sharply with increasing content of MPEG in the polymer by addition of NaCl: $\Delta LCST(T_{1.0M} - T_{0M})$ is −27.0 °C for polymer **1**, −19.2 °C for polymer **3**, and −12.0 °C for polymer **4**, as shown in Table 1. Such an observation indicates that change in the LCST of the polymers by a water structure maker, NaCl, seems to be due to decrease in the interaction between the hydrophilic MPEG in the polymer and water molecules. As a result, the higher content of hydrophilic MPEG in the polymer leads to higher sensitivity to the salt concentration. A similar trend was observed in poly(diacetone acryla-

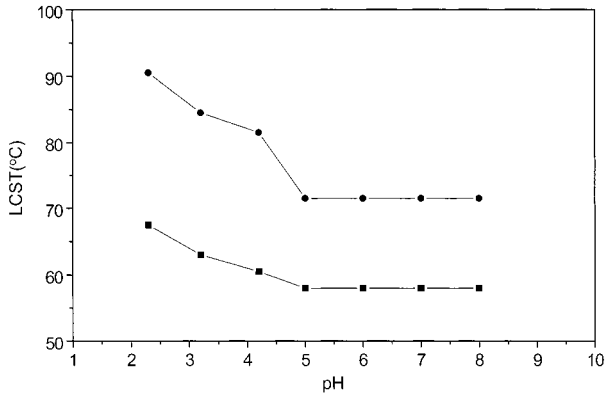


Figure 7. The pH effect on LCST of polymer **3** (●) and polymer **10** (■) solutions.

mide-*co*-acrylamide) studied by Taylor and co-workers.[26] They pointed out that the cloud point of the copolymer decreased by addition of NaCl more sharply with increasing content of hydrophilic acrylamide in the copolymer. The change of the LCST of the polymers by NaCl is also dependent on the structure of the ester groups, but all the polymers reveal the salting-out effect. When a polymer has more hydrophilic ester groups, the LCST was found to change more drastically depending on the salt concentration: $\Delta LCST(T_{1.0M} - T_{0M})$ is −25.7 °C for methyl ester (polymer **5**), −19.2 °C for ethyl ester (polymer **3**), and −13.7 °C for benzyl ester (polymer **6**), as shown in Table 1.

We have examined the pH dependence of the LCST of polymers **3** and **10** in acetate buffer solutions at different pH, and the results are presented in Figure 7. The LCST of polymers with the same mole ratios of MPEG and amino acid ethyl ester exhibit salting-in effects in the acidic media at pH < 4 and salting-out effects in other buffer solutions. Furthermore, in low-pH buffer solutions, the LCST changed quite sharply, which was more prominent for polymer **3** than polymer **10**, but in the buffer solutions at pH > 5, the LCST was independent of the pH change. In the acidic region, the degree of ionization of the amine groups of the amino acid esters of the present polymers is presumed to increase with decreasing pH of the buffer solution, resulting in increase of their LCST. Beltran et al.[27] have studied pH-dependent swelling properties of *N*-isopropylacrylamide (NIPA) copolymer gels. It was pointed out that the swelling capacity of NIPA/2-(dimethylamino)-ethyl methacrylate copolymer gel increased with decreasing pH whereas that of the NIPA/sodium acrylate copolymer gel increased with increasing pH. It is also known that the pH-dependent swelling capacity of the copolymer gels decreases with increasing hydrophobicity.[28,29]

Conclusions

Hydrolytic degradation of the present thermosensitive poly(organophoaphazenes) with MPEG and amino acid ester side groups was affected by the structures of amino acids and ester groups, mole ratio of substituents, and pH and temperature of the buffer solutions. The polymers substituted with α-amino acid esters were hydrolyzed more rapidly than that with the *β*-amino acid ester. The higher content of the amino acid ester in the polymer gave rise to more rapid hydrolysis. The structure of ester groups also significantly affected the polymer degradation, and the order of sensitivity to polymer degradation was methyl > ethyl > benzyl esters. Hydrolysis of the polymers occurred more rapidly in the acidic and basic solutions than in the neutral solution. These results are consistent with the carboxylic acid-catalyzed degradation, and a pathway to the degradation of present thermosensitive polyphosphazenes was proposed. It seems possible to control the hydrolysis rate of the polymers by proper design of the structure of the pendent groups.

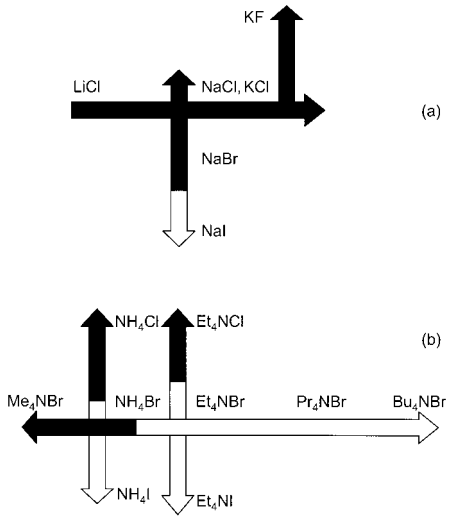


Figure 8. The salting-in (unshaded arrows) and salting-out (shaded arrows) effects of the inorganic (a) and organic salts (b).

The LCST of the aqueous solutions of the present thermosensitive polymers was found to be affected by kinds of salts, composition of substituents, the structures of amino acids and ester groups, and the chain length of MPEG. The effect of the inorganic and organic salts on the LCST of the present polymers is summarized in Figure 8. Most of the inorganic salts play water structure makers and therefore increase the LCST of the polymer solutions except for NaI, which decreases the LCST. In the case of tetraalkylammonium halides which are organic salts, the chain length of their alkyl groups plays an important role: an increase in the chain length of the alkyl group (especially, tetrabutylammonium cation) leads to an increase in the LCST of the polymer solutions. NaCl plays as water structure maker, and its effect on the LCST was dependent on the polymer structure: Generally, NaCl exhibits a stronger effect on the more hydrophilic polymers, and as a result, the higher content of MEPG as well as the more hydrophilic ester groups causes a decrease in the LCST more drastically. The pH of the buffer solution also affected the LCST of the polymers: the LCST of the polymers increased in the buffer solution at pH < 4 but decreased in other media. Such unique phase transition behaviors may offer a wide range of potential applications such as isolation of biomolecules, permeation control of biomembrane, and ion-sensitive biosensors.

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