A New Class of Biodegradable Thermosensitive Polymers. I. Synthesis and Characterization of Poly(organophosphazenes) with Methoxy-Poly(ethylene glycol) and Amino Acid Esters as Side Groups

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ABSTRACT: Novel thermosensitive poly(organophosphazenes) bearing methoxy-poly(ethylene glycol) (MPEG) and amino acid esters as substituents have been synthesized, and their lower critical solution temperature (LCST) was investigated. Differential scanning calorimetry (DSC) has shown that some of the polymers exhibit crystallinity, which is probably induced by the MPEG side chain of the polymers. Most of the polymers show their LCSTs in the range of 25.0−98.5 °C, depending on several factors such as mole ratio of the substituents, molecular weight of the MPEG, and kinds of amino acids and esters. The more hydrophilic composition of the polymers offers the higher LCST. The LCST of the polymers exhibits almost concentration-independent behavior in the range of 3−30 wt % of the polymers in aqueous solution.

Introduction

Thermosensitive polymers have been studied extensively over the past decade.[1−8] Many thermosensitive polymer solutions exhibit a lower critical solution temperature (LCST), defined as the critical temperature at which a polymer solution undergoes phase transition from a soluble to an insoluble state when the temperature is raised. The main mechanism of a thermally induced phase transition of aqueous polymer solutions is a drastic change in the hydrophilic−hydrophobic balance of the polymer due to the release of water bound to the polymer. These unique properties of the thermosensitive polymers make them useful for application to many fields, such as membranes,[3] drug delivery systems,[4,5] cell culture,[6] isolation of biomolecules,[7] and enzyme activity control.[8]

The homopolymer or copolymers of *N*-isopropylacrylamide are representative thermosensitive polymers.[9,10] Their LCST is around body temperature (∼32 °C), and therefore, application of these polymers as drug delivery materials has been extensively studied. However, their application to a drug delivery system is difficult because these polymers are toxic and nonbiodegradable. Biodegradability of polymeric materials is one of the most important factors for a polymer-based drug delivery system. Most of the thermosensitive polymers including the graft-copolymer and block copolymer of poly(ethylene oxide) and poly(vinyl alcohol) derivatives are known to be nonbiodegradable.[11] Several kinds of thermosensitive poly(organophosphazenes) bearing alkyl ether side groups have been studied as electrolyte material, but these polymers are also nondegradable.[12−14] Recently, biodegradable thermosensitive polymers, poly(ethylene oxide) and poly(L-lactic acid) block copolymers, have been reported as an injectable drug delivery material.[15] Aqueous solutions of these copolymers exhibit a temperature-dependent reversible sol−gel transition.

In the present work, aiming to design a biodegradable thermosensitive polymer which could be useful for a drug delivery system, we have synthesized and characterized poly(organophosphazenes) bearing side groups of poly(ethylene glycol) and amino acid esters.

Experimental Section

Materials. Hexachlorocyclotriphosphazene (Aldrich) was used without purification. Methyl, ethyl, and benzyl esters of glycine and L-aspartic acid and ethyl esters of alanine and L-glutamic acid were prepared by the literature method.[16] Diethyl aminomalonate (Aldrich) was used as received. Methoxy-poly(ethylene glycol) with molecular weights of 350 and 750 were dried azeotropically with benzene, followed by vacuum-drying, and then stored over molecular sieve 4A. Tetrahydrofuran (THF) was dried by boiling at reflux over sodium metal and distilled under a nitrogen atmosphere.

Instruments. Elemental analysis was carried out with Fisons 1108 CHNS microanalyzer and Polyscan 61E ICP. ^{1}H 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. Thermal analysis of the polymers was carried out using Dupont DSC 2100 TA Instruments. The sample was heated at a rate of 5 °C/min in the range of −100 to +40 °C. Gel-permeation chromatography was carried out 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 inline 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, and 95 000) were used as standards to calibrate the column.

Measurement of LCST. The phase transition of the aqueous solution of a polymer (0.1−30 wt %) was detected visually in a closed glass tube, and the temperature was controlled by immersion of the glass tube in an oil bath. The LCST was identified as the temperature at which the solution became turbid. The LCST was also determined by UV−vis spectroscopy: The polymer was dissolved in distilled water to a given concentration, and the polymer solution was poured into a cell. The cell holder in the spectrophotometer was thermally controlled by heating and cooling. The absorbance at 600 nm was monitored by increasing the solution temperature, and the LCST was defined as the temperature where 50% of the absorbance change occurred.

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Synthesis of $[NP(MPEG)_x(AME)_{2-x}]_n$ [MPEG: methoxy-poly(ethylene glycol); AME: amino acid ester]. Poly(dichlorophosphazene) was prepared as described previously.[17] The typical synthetic procedure is as follows. The sodium salt of methoxy-poly(ethylene glycol) (M_w = 350, MPEG350) was prepared by reaction of MPEG350 (1.8 g, 5.2 mmol) with 1.5 equiv of sodium metal in THF (150 mL) at refluxing temperature for 2 days. After the resultant solution was filtered to remove excess sodium, the filtrate solution was added slowly to poly(dichlorophosphazene) (2.0 g, 17.3 mmol) dissolved in THF (100 mL). The reaction mixture was stirred for 5 h at room temperature. Meanwhile, glycine ethyl ester hydrochloride (8.2 g, 58.7 mmol) was suspended in dry THF (100 mL) containing 4 equiv of triethylamine. The polymer solution was transferred to the glycine ethyl ester solution, which was stirred for 2 days at 50 °C. The reaction mixture was filtered, and after the filtrate was concentrated, it was poured into a mixed solvent of diethyl ether and hexane (1:1) to obtain a precipitate, which was reprecipitated twice in the same solvent system. The polymer was dissolved in a small amount of methanol, which was dialyzed in distilled water for 2 days. The dialyzed solution was freeze-dried to obtain the polymer **1**. The other polymers were prepared analogously using different substituents and different mole ratios.

$[NP(MPEG350)_{0.31}(GlyEt)_{1.69}]_n$ (1). Yield: 87%. ^{31}P NMR (acetone-d_6), δ (ppm): 24.09. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(m, 3H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 4H). Elem. anal. (%) calcd: C, 40.71; H, 6.89; N, 13.61; P, 10.63. Found: C, 40.00; H, 6.65; N, 13.90; P, 10.40.

$[NP(MPEG350)_{0.48}(GlyEt)_{1.52}]_n$ (2). 8.6 mmol of MPEG350 and 51.8 mmol of glycine ethyl ester were used. Yield: 89%. ^{31}P NMR (acetone-d_6), δ (ppm): 23.87. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(m, 3H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 4H). Elem. anal. (%) calcd: C, 38.20; H, 6.90; N, 10.12; P, 9.07. Found: C, 39.80; H, 6.87; N, 10.20; P, 9.05.

$[NP(MPEG350)_{0.58}(GlyEt)_{1.42}]_n$ (3). 12.9 mmol of MPEG350 and 43.2 mmol of glycine ethyl ester were used. Yield: 77%. ^{31}P NMR (acetone-d_6), δ (ppm): 23.77. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(m, 3H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 4H). Elem. anal. (%) calcd: C, 42.89; H, 7.49; N, 9.26; P, 8.19. Found: C, 42.30; H, 7.27; N, 9.29; P, 8.34.

$[NP(MPEG350)_{0.99}(GlyEt)_{1.01}]_n$ (4). 17.3 mmol of MPEG350 and 34.5 mmol of glycine ethyl ester were used. Yield: 53%. ^{31}P NMR (acetone-d_6), δ (ppm): 23.03. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(m, 3H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 4H). ^{13}C NMR (D_2O), δ (ppm): 173.38, 71.18, 69.28, 65.3, 58.22, 43.05, 13.87. Elem. anal. (%) calcd: C, 45.73; H, 7.87; N, 6.16; P, 6.46. Found: C, 46.05; H, 7.94; N, 6.24; P, 6.23.

$[NP(MPEG350)_{1.12}(GlyEt)_{0.88}]_n$ (5). 25.9 mmol of MPEG350 and 34.5 mmol of glycine ethyl ester were used. Yield: 48%. ^{31}P NMR (acetone-d_6), δ (ppm): 23.01, 18.44. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(m, 3H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 4H). Elem. anal. (%) calcd: C, 46.56; H, 8.48; N, 5.79; P, 5.70. Found: C, 46.97; H, 8.57; N, 5.76; P, 5.72.

$[NP(MPEG350)_{1.42}(GlyEt)_{0.58}]_n$ (6). 30.2 mmol of MPEG350 and 34.5 mmol of glycine ethyl ester were used. Yield: 80%. ^{31}P NMR (acetone-d_6), δ (ppm): 22.35, 18.02. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(m, 3H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 4H). Elem. anal. (%) calcd: C, 46.98; H, 8.12; N, 4.18; P, 5.43. Found: C, 46.65; H, 8.88; N, 4.11; P, 5.30.

$[NP(MPEG350)_{1.71}(GlyEt)_{0.29}]_n$ (7). 33.7 mmol of MPEG350 and 17.3 mmol of glycine ethyl ester were used. Yield: 88%. ^{31}P NMR (acetone-d_6), δ (ppm): 14−22. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(m, 3H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 4H). Elem. anal. (%) calcd: C, 47.84; H, 8.32; N, 2.68; P, 4.60. Found: C, 48.0; H, 8.51; N, 2.92; P, 4.47.

$[NP(MPEG350)_{1.85}(GlyEt)_{0.15}]_n$ (8). 37.1 mmol of MPEG350 and 17.3 mmol of glycine ethyl ester were used. Yield: 70%. ^{31}P NMR (acetone-d_6), δ (ppm): 14−21. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(m, 3H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 4H). Elem. anal. (%) calcd: C, 47.99; H, 8.37; N, 2.27; P, 4.36. Found: C, 48.50; H, 8.54; N, 2.39; P, 4.31.

$[NP(MDEG350)_{1.03}(GlyMe)_{0.97}]_n$ (9). 17.3 mmol of MPEG350 and 34.5 mmol of glycine methyl ester were used. Yield: 62%. ^{31}P NMR (acetone-d_6), δ (ppm): 23.01. ^{1}H NMR (D_2O), δ (ppm): 3.4(s, 3H), 3.6−3.9(b, 29H), 4.0−4.4(b, 4H). Elem. anal. (%) calcd: C, 43.78; H, 7.78; N, 5.76; P, 6.47. Found: C, 43.48; H, 7.74; N, 6.09; P, 6.36.

$[NP(MPEG350)_{1.00}(GlyBz)_{1.00}]_n$ (10). 17.3 mmol of MPEG350 and 34.5 mmol of glycine benzyl ester were used. Yield: 80%. ^{31}P NMR (acetone-d_6), δ (ppm): 23.88. ^{1}H NMR (D_2O), δ (ppm): 3.4(s, 3H), 3.4−3.8(b, 26H), 4.0−4.4(b, 4H), 5.2−5.3(b, 2H), 6.9−7.2(b, 5H). Elem. anal. (%) calcd: C, 51.44; H, 7.16; N, 6.51; P, 5.45. Found: C, 51.30; H, 7.09; N, 6.31; P, 5.33.

$[NP(MPEG750)_{1.09}(GlyEt)_{0.91}]_n$ (11). 17.3 mmol of methoxy-poly(ethylene glycol) (M_w = 750, MPEG750) and 34.5 mmol of glycine ethyl ester were used. Yield: 76%. ^{31}P NMR (acetone-d_6), δ (ppm): 23.11. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(m, 3H), 3.4(s, 3H), 3.6−3.9(b, 62H), 4.0−4.4(b, 4H). Elem. anal. (%) calcd: C, 49.60; H, 9.11; N, 3.13; P, 3.46. Found: C, 50.28; H, 9.05; N, 3.22; P, 3.61.

$[NP(MPEG350)_{1.00}(AlaEt)_{1.00}]_n$ (12). 17.3 mmol of MPEG350 and 34.5 mmol of L-alanine ethyl ester were used. Yield: 76%. ^{31}P NMR (acetone-d_6), δ (ppm): 22.06. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.5(m, 3H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 5H). ^{13}C NMR (D_2O), δ (ppm): 175.94, 71.25, 69.85, 64.10, 61.42, 58.28, 50.01, 20.89, 13.99. Elem. anal. (%) calcd: C, 47.09; H, 8.10; N, 6.51; P, 6.07. Found: C, 47.14; H, 8.30; N, 6.62; P, 6.08.

$[NP(MPEG350)_{0.97}(MalEt_2)_{1.03}]_n$ (13). 17.3 mmol of MPEG350 and 34.5 mmol of aminomalonic acid ethyl ester were used. Yield: 72%. ^{31}P NMR (acetone-d_6), δ (ppm): 21.07. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(m, 6H), 3.4(s, 3H), 3.6−3.9-(b, 26H), 4.0−4.4(b, 7H). ^{13}C NMR (D_2O), δ (ppm): 169.18, 71.27, 69.86, 64.77, 63.24, 58.31, 13.81. Elem. anal. (%) calcd: C, 45.11; H, 7.64; N, 6.26; P, 5.81. Found: C, 44.99; H, 7.57; N, 6.33; P, 5.89.

$[NP(MPEG350)_{0.66}(AspEt_2)_{1.33}]_n$ (14). 12.1 mmol of MPEG350 and 44.9 mmol of L-aspartic acid ethyl ester were used. Yield: 62%. ^{31}P NMR (acetone-d_6), δ (ppm): 22.0. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(m, 6H), 2.9−3.2(b, 2H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 7H). Elem. anal. (%) calcd: C, 44.70; H, 7.29; N, 7.24; P, 6.69. Found: C, 44.80; H, 7.31; N, 7.17; P, 6.96.

$[NP(MPEG350)_{1.01}(AspEt_2)_{0.99}]_n$ (15). 17.3 mmol of MPEG350 and 34.5 mmol of L-aspartic acid ethyl ester were used. Yield: 78%. ^{31}P NMR (acetone-d_6), δ (ppm): 21.96. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(m, 6H), 2.9−3.2(b, 2H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 7H). ^{13}C NMR (D_2O), δ (ppm): 173.33, 172.04, 71.19, 69.79, 64.39, 62.39, 58.29, 50.09, 39.0, 13.89. Elem. anal. (%) calcd: C, 46.38; H, 7.79; N, 5.01; P, 5.57. Found: C, 46.32; H, 7.67; N, 5.18; P, 5.59.

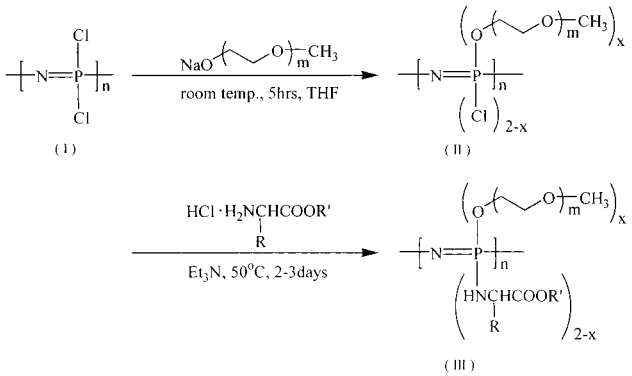
$[NP(MPEG350)_{0.99}(AspMe_2)_{1.01}]_n$ (16). 17.3 mmol of MPEG350 and 34.5 mmol of L-aspartic acid methyl ester were used. Yield: 80%. ^{31}P NMR (acetone-d_6), δ (ppm): 21.95. ^{1}H NMR (D_2O), δ (ppm): 2.9−3.2(b, 2H), 3.4(s, 3H), 3.6−3.9(b, 32H), 4.0−4.4(b, 3H). Elem. anal. (%) calcd: C, 44.71; H, 7.71; N, 5.47; P, 6.13. Found: C, 45.03; H, 7.80; N, 5.67; P, 6.21.

$[NP(MPEG350)_{1.03}(AspBz_2)_{0.97}]_n$ (17). 17.3 mmol of MPEG350 and 34.5 mmol of L-aspartic acid benzyl ester were used. Yield: 29%. ^{31}P NMR (acetone-d_6), δ (ppm): 22.41. ^{1}H NMR (D_2O), δ (ppm): 2.9−3.2(b, 2H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 3H), 5.2−5.3(b, 4H), 6.9−7.2(b, 10H). Elem. anal. (%) calcd: C, 55.40; H, 7.58; N, 4.38; P, 5.45. Found: C, 56.51; H, 7.44; N, 4.27; P, 5.51.

$[NP(MPEG750)_{1.09}(AspEt_2)_{0.91}]_n$ (18). 17.3 mmol of MPEG750 and aspartic 34.5 mmol of L-aspartic acid ethyl ester were used. Yield: 55%. ^{31}P NMR (acetone-d_6), δ (ppm): 21.82. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(b, 6H), 2.9−3.2(b, 2H), 3.4-(s, 3H), 3.6−3.9(b, 62H), 4.0−4.4(b, 7H). Elem. anal. (%) calcd: C, 48.99; H, 8.45; N, 3.27; P, 2.93. Found: C, 48.90; H, 8.44; N, 3.34; P, 3.10.

$[NP(MPEG350)_{0.20}(GluEt_2)_{1.43}(OH)_{0.37}]_n$ (19). 10.4 mmol of MPEG350 and 41.4 mmol of L-glutamic acid ethyl ester were used. Yield: 55%. ^{31}P NMR (acetone-d_6), δ (ppm): 21.24. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(b, 6H), 2.9−3.2(b, 2H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 7H). Elem. anal. (%) calcd: C, 41.64; H, 7.16; N, 8.57; P, 8.88. Found: C, 41.50; H, 6.77; N, 8.61; P, 8.81.

Scheme 1



m = 7 (MPEG350), 16 (MPEG750)

R = H (Gly), CH_3 (Ala), COOR' (Mal), CH_2COOR' (Asp), CH_2CH_2COOR' (Glu)

R' = CH_3 (Me), C_2H_5 (Et), $CH_2C_6H_5$ (Bz)

$[NP(MPEG350)_{1.09}(GluEt_2)_{0.79}(OH)_{0.12}]_n$ (20). 17.3 mmol of MPEG350 and 34.5 mmol of L-glutamic acid ethyl ester were used. Yield: 60%. ^{31}P NMR (acetone-d_6), δ (ppm): 22.02. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(b, 6H), 2.0−2.2(b, 2H), 2.5−2.6(b, 2H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 7H). ^{13}C NMR (D_2O), δ (ppm): 174.57, 71.23, 69.81, 65.11, 61.55, 58.27, 53.72, 30.36, 29.1, 13.96. Elem. anal. (%) calcd: C, 46.0; H, 8.0; N, 4.47; P, 5.49. Found: C, 46.90; H, 7.89; N, 4.58; P, 5.49.

$[NP(MPEG350)_{0.59}(GluEt_2)_{0.40}(GlyEt)_{1.01}]_n$ (21). After poly(dichlorophosphazene) was reacted with the sodium salt of MPEG350 (8.63 mmol) and L-glutamic acid ethyl ester (12.94 mmol) in the same way as that used for polymer **1**, glycine ethyl ester (25.89 mmol) was added to the reaction mixture, which was stirred for 2 days at 50 °C. Purification of the polymer was performed by the same method as that used for polymer **1**. Yield: 85%. ^{31}P NMR (acetone-d_6), δ (ppm): 20.42. ^{1}H NMR (D_2O), δ (ppm): 1.2−1.4(b, 9H), 2.0−2.2(b, 2H), 2.5−2.6(b, 2H), 3.4(s, 3H), 3.6−3.9(b, 26H), 4.0−4.4(b, 9H). ^{13}C NMR (D_2O), δ (ppm): 174.06, 173.61, 71.29, 70.60, 64.33, 61.22, 58.26, 53.68, 43.17, 30.34, 13.99. Elem. anal. (%) calcd: C, 45.19; H, 7.77; N, 7.70; P, 7.07. Found: C, 45.40; H, 7.49; N, 7.68; P, 7.31.

$[NP(GlyEt)_2]_n$ and $[NP(MPEG350)_2]_n$. The homopolymers of the glycine ethyl ester and MPEG350 were prepared by the literature method.[12,18]

Results and Discussion

Synthesis and Characterization. The polymers were synthesized by the synthetic method in Scheme 1. Poly(dichlorophosphazene) (I) dissolved in THF was reacted with the sodium salt of MPEG to yield polymer (II), and an excess amount of amino acid esters was then reacted to yield polymer (III). Many different copolymers were obtained by varying the amino acid esters and the MPEG and by changing the mole ratio of the two substituents. The polymers obtained were characterized by means of multinuclear (^{1}H, ^{13}C, ^{31}P) NMR spectroscopies, DSC, GPC, UV, and elemental analysis. The ^{1}H and ^{13}C NMR spectra of polymer **4** are shown in Figure 1. The mole ratio of MPEG and the glycine ethyl ester of polymer **4** was calculated from the integration ratio between the methyl protons of the glycine ethyl ester and the methoxy protons of the MPEG appearing at 1.3 and 3.4 ppm, respectively. The mole ratio of the MPEG and the amino acid ester in other polymers was

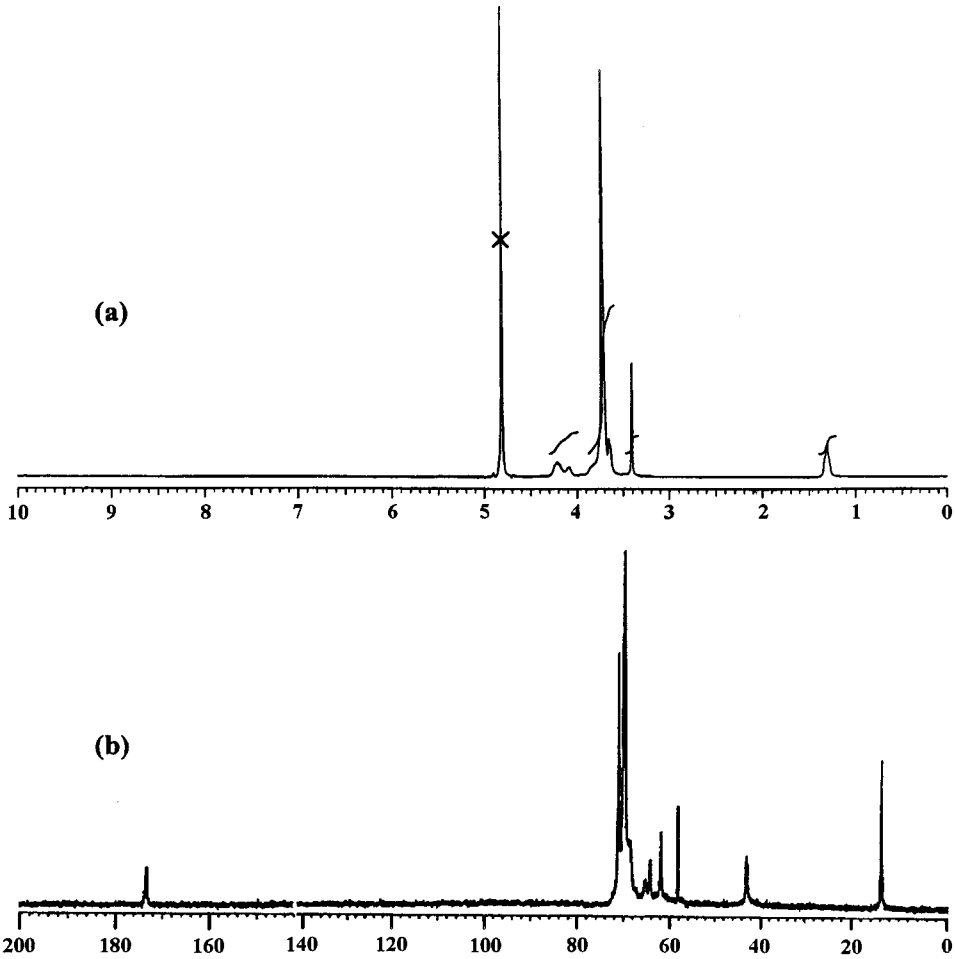
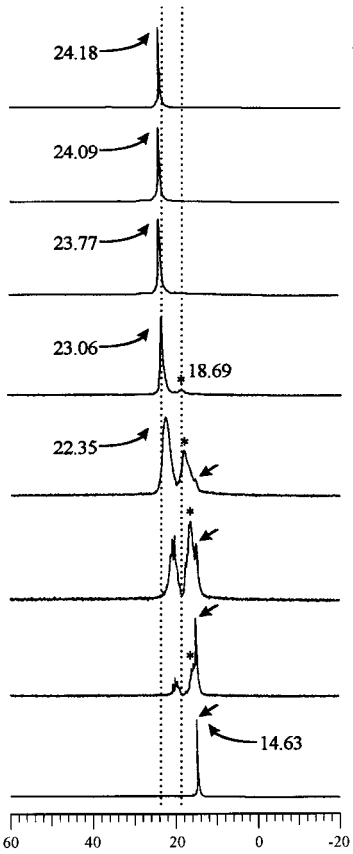


Figure 1. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of polymer **4**.



polymers	mole ratio	
	MPEG350	GlyEt
	0	2.00
1	0.31	1.69
3	0.58	1.42
4	0.99	1.01
6	1.42	0.58
7	1.71	0.29
8	1.85	0.15
	2.00	0

Figure 2. ^{31}P NMR spectra of the polymers with various mole ratios of MPEG and glycine ethyl ester.

estimated in the same way. Figure 2 shows the ^{31}P NMR spectra of poly(organophosphazenes) substituted by MPEG350 and the glycine ethyl ester depending on the mole ratio of the two substituents. The homopolymers of the glycine ethyl ester and MPEG350 each show a single sharp peak at 24.18 and 14.63 ppm, respectively. The polymer bearing the almost equimolar glycine ethyl ester and MPEG shows a sharp peak at 23.06 ppm with a slight tail and a very small peak at 18.69 ppm. The peak at 23.06 ppm seems to be ascribed to the phosphorus resonances of the main −N−P−O− unit and a small amount of the −N−P−N− unit. The signal at 18.69 ppm was almost 4 ppm downfield shifted compared to that of the −O−P−O− unit in the MPEG homopolymer. This may be attributed to the influence by the adjacent −N−P−O− unit. This indicates that the initial substitution by MPEG leads to mainly monosubstituted −O−P−Cl units, after which the chlorine is fully replaced by the glycine ethyl ester. The peak at 18.69 ppm became larger and upfield shifted with increasing content of MPEG, whereas that at 23.06 ppm became smaller and finally disappeared in the polymers **7** and **8** and the MPEG homopolymer. The polymer **6** with a 1.42:0.58 mole ratio of MPEG/GlyEt began to show a signal at 14.63 ppm corresponding to that of the MPEG homopolymer. This signal grew with an increasing ratio of MPEG to GlyEt.

Figure 3 shows the ^{31}P NMR spectra of the copolymers substituted with MPEG and various amino acids. The peak sharpness in the spectra was found to be dependent on the bulkiness of the substituents. A sharp peak was observed when relatively small amino acid esters, such as glycine, alanine, and aminomalonic acid were used as the second substituents. Such a result implies

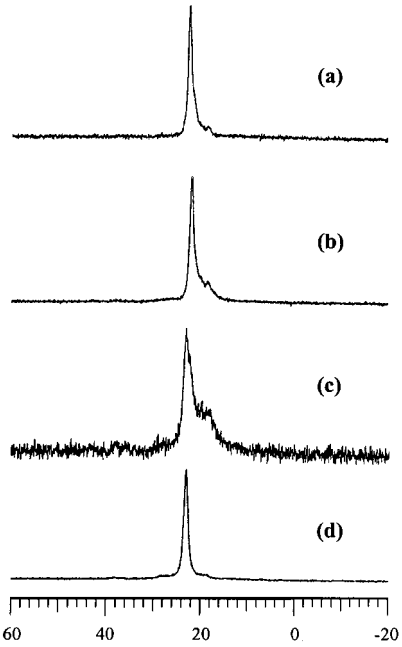


Figure 3. ^{31}P NMR spectra of (a) polymer **12**, (b) polymer **15**, (c) polymer **20**, and (d) polymer **21**.

that the chlorine atom of poly(dichlorophosphazene) was completely substituted stepwise by MPEG and amino acid esters. On the other hand, a broad peak was observed in polymer **20** when L-glutamic acid ester was the cosubstituent. It appears that the chlorine atom of poly(dichlorophosphazene) was not completely replaced during the second substitution reaction by L-glutamic acid ethyl ester because of its bulkiness, and the residual chlorine was hydrolyzed by water during the purification process. From the results of elemental analysis, the mole fraction of the hydroxy group in polymers **19** and **20** were estimated to be 0.37 and 0.12, respectively. In this case, glycine ethyl ester was employed as a third substituent in order to replace the residual chlorine after two-step substitutions with MPEG and L-glutamic acid ester. Figure 3d shows one sharp peak, indicating that the residual chlorine that remained after the second substitution by L-glutamic acid ester was completely replaced by the smaller glycine ethyl ester.

All of the copolymers substituted by MPEG and amino acid esters were obtained as pale yellow viscoelastic solids, which are fairly soluble in most organic solvents and very soluble in water below LCST.

Most of the polymers had glass-transition temperatures (T_g) below room temperature, as shown in Table 1. The lowest T_g values correspond to higher content and long chain of MPEG side groups. Such results indicate that the high mobility of the phosphazene backbone is not much hindered by the substituents and that the MPEG disrupts the polymer chain stacking to some extent. Figure 4 shows the DSC curves of the copolymers substituted by MPEG350 and glycine ethyl ester. The crystallization temperature (T_c) was observed in the polymers **5** and **6** with 1.12:0.88 and 1.42:0.58 mole ratios of MPEG/GlyEt, respectively. Polymers **7** and **8** were found to exhibit T_c only when the polymers were heated and quenched (data not shown). It appears that the crystallinity of the polymers is induced by the MPEG side group, and therefore, the polymer with less than 1.0 equiv of MPEG/P═N unit seems to show no

Table 1. Characteristics of Poly(organophosphazenes)

polymer	formula	T_g (°C)	T_m (°C)	LCST (°C)	M_w ($\times 10^{-4}$)[a]
1	$[NP(MPEG350)_{0.31}(GlyEt)_{1.69}]_n$	−36		35.0	
2	$[NP(MPEG350)_{0.48}(GlyEt)_{1.52}]_n$	−51		58.0	1.57
3	$[NP(MPEG350)_{0.58}(GlyEt)_{1.42}]_n$	−56		64.5	1.77
4	$[NP(MPEG350)_{0.99}(GlyEt)_{1.01}]_n$	−68		77.5	3.48
5	$[NP(MPEG350)_{1.12}(GlyEt)_{0.88}]_n$	−71	−17	83.7	3.11
6	$[NP(MPEG350)_{1.42}(GlyEt)_{0.58}]_n$	−72	−15	93.2	4.73
7	$[NP(MPEG350)_{1.71}(GlyEt)_{0.29}]_n$	−72	−7		2.94
8	$[NP(MPEG350)_{1.85}(GlyEt)_{0.15}]_n$	−74	−6		3.45
9	$[NP(MPEG350)_{1.03}(GlyMe)_{0.97}]_n$	−79		88.5	3.08
10	$[NP(MPEG350)_{1.00}(GlyBz)_{1.00}]_n$	−40		49.5	2.13
11	$[NP(MPEG750)_{1.09}(GlyEt)_{0.91}]_n$	−64	24	98.5	4.14
12	$[NP(MPEG350)_{1.00}(AlaEt)_{1.00}]_n$	−67		67.0	3.58
13	$[NP(MPEG350)_{0.97}(MalEt_2)_{1.03}]_n$	−64		65.5	2.24
14	$[NP(MPEG350)_{0.66}(AspEt_2)_{1.33}]_n$	−77		38.5	
15	$[NP(MPEG350)_{1.01}(AspEt_2)_{0.99}]_n$	−67		60.2	4.40
16	$[NP(MPEG350)_{0.99}(AspMe_2)_{1.01}]_n$	−58		84.3	1.79
17	$[NP(MPEG350)_{1.03}(AspBz_2)_{0.97}]_n$	−51		33.8	
18	$[NP(MPEG750)_{1.09}(AspEt_2)_{0.91}]_n$	−68	19	75.0	4.23
19	$[NP(MPEG350)_{0.20}(GluEt_2)_{1.43}(OH)_{0.37}]_n$	−42		25.2	
20	$[NP(MPEG350)_{1.09}(GluEt_2)_{0.79}(OH)_{0.12}]_n$	−70		66.5	2.21
21	$[NP(MPEG350)_{0.59}(GluEt_2)_{0.40}(GlyEt)_{1.01}]_n$	−65		53.6	3.58

[a] Molecular weight of the polymers was calculated by GPC using a cosolvent of distilled water and acetonitrile (4:1) and those of polymers **1**, **14**, **17**, and **19** could not be examined due to their low LCST.

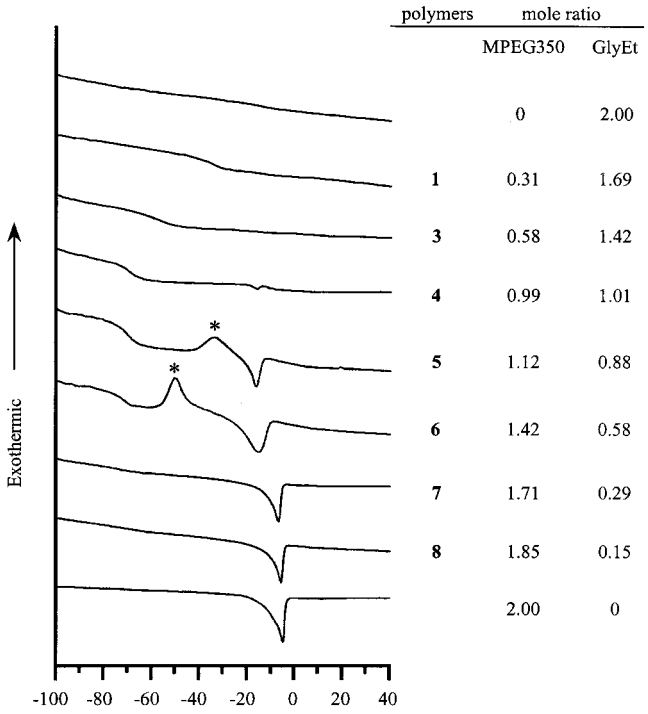


Figure 4. DSC curves of the polymers with various mole ratios of MPEG and glycine ethyl ester. T_c is indicated by *.

crystallinity. Similarly, the melting transition temperature (T_m) was clearly influenced by the contents and the length of the MPEG as observed in those polymers with more than 1.0 mol content of MPEG350 (polymer **5**−**8**) and MPEG750 (polymer **11**, **18**) per unit, as shown in Table 1. Higher content and longer chains of MPEG increased the T_m. As the MPEG content increased from 1.12 mol (polymer **5**) to 1.85 mol (polymer **8**) per unit, T_m increased from −17 to −6 °C. The MPEG chain length had a larger influence on T_m. As the MPEG molecular weight increased from 350 (polymer **5**) to 750 (polymer **11**), T_m increased from −17 to 24 °C. The amino acid esters are smaller than MPEG chains, but when the mole fraction of the amino acid ester is larger than that of MPEG, the amino acid ester chain seems to interfere with the orientation of the MPEG side chains. As a result, the polymers with less than 1.0 mol of the MPEG contents/unit are presumed to show no T_m. Such a thermal behavior is similar to that of ethylene oxide based polyphosphazene derivatives.[13,19]

Thermosensitivity. The phase transition of thermosensitive polymers in aqueous solution is attributed to a change in the hydrophilic−hydrophobic balance of the polymers with respect to their interaction through hydrogen bonding between the polymer and water molecules. Below the LCST, the hydrogen-bonding interactions predominate over the hydrophobic interaction of the polymers, which results in water-soluble solutions. On the other hand, when the hydrogen-bonding interactions are weakened and the hydrophobic interaction of the polymer increases, the polymer precipitates from the solution. Therefore, increasing the hydrophilicity of the polymer causes an increase in the LCST of the polymer solution. Such a tendency was also observed in our polymer systems. The LCST of the polymers was measured in an aqueous solution by a melting point apparatus and UV spectroscopy. As shown in Table 1, most of the polymer solutions exhibit the LCST in a range of 25.2 to 98.5 °C. For the polymers bearing MPEG and glycine ethyl ester groups, the LCST increases with increasing MPEG content and the polymers containing over 1.7 mol of MPEG/unit show no LCST because of their very high hydrophilicity. The LCST of the polymers with almost equimolar substituents was influenced by different kinds of esters. The more hydrophobic ester group afforded the lower LCST. For example, the LCST of the polymers with methyl, ethyl, and benzyl ester of glycine were 88.5, 77.5, and 49.5 °C, respectively. Longer chains of MPEG offered a higher LCST, as demonstrated by polymers **4** and **11** where the LCST were 78 and 98 °C, respectively. These results are consistent with the studies of polyphosphazenes substituted with various alkyl ethers by Allcock and co-workers.[13] They have noted that increasing the alkyl ether chain length increased opportunities for hydration of alkyl ether groups, resulting in increased LCST. Similarly, Schacht and co-workers have reported the synthesis and hydrolytic properties of poly(phosphazene)-poly(ethylene oxide) copolymers without LCST properties.[18,19] They employed as a substituent

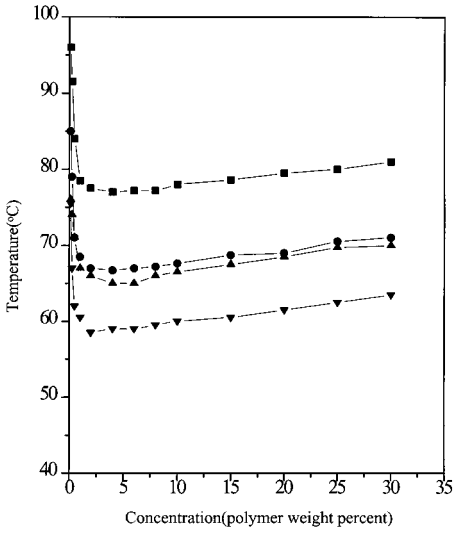


Figure 5. Concentration dependent LCST behaviors of polymers **4** (■), **12** (●), **13** (▲), and **15** (▼).

poly(ethylene oxide) with a molecular weight of 750, 2000, or 5000 which is assumed to be too hydrophilic to exhibit a LCST. A similar trend was observed in our polymers substituted by MPEG and L-aspartic acid esters. The LCST of the polymers **14** and **15** with 0.66: 1.34 and 1.01:0.99 mole ratios of MPEG350/AspEt were 38.5 and 60.2 °C, respectively. The LCST of polymers with a similar mole ratio of MPEG/methyl (**16**), ethyl (**17**), and benzyl (**18**) esters of L-aspartic acid were 84.3, 60.2, and 33.8 °C, respectively. The effects of the mole ratio of the two substituents and ester groups on the LCST were larger for the polymers with MPEG and L-aspartic acid than for the polymers with MPEG and glycine. This result seems to be due to the difference in hydrophobicity between the di- and monocarboxylic acids. The LCST of the polymers was also dependent on the kinds of amino acid: the more hydrophobic amino acid afforded the lower LCST of the polymer, i.e., the LCST decreased in the order of glycine, alanine, aminomalonic acid, L-aspartic acid, and L-glutamic acid ethyl esters. Such results imply that the LCST of the polymers can be controlled by appropriately choosing several factors such as the mole ratio of the two substituents, length of MPEG, kind of amino acid, and ester groups.

The concentration-dependent LCST of the polymers was determined in the concentration range of 0.1−30 wt % of the polymers in aqueous solution. Figure 5 shows the LCST values for several polymers at various concentrations. The polymers precipitated at all concentrations, and no significant differences in the concentration-dependent LCST behavior were found among the polymers. The LCST of the polymers was found to be almost independent in the range of 3−30 wt % of the polymers but increase at lower concentrations as, observed for other thermosensitive polymers.[3,13]

Conclusions

Novel biodegradable thermosensitive poly(organophosphazenes) bearing side groups of MPEG and amino acid esters have been synthesized, and their LCST behavior was investigated. The LCST of the polymers was affected by several factors such as composition of substituents, chain length of MPEG, and kinds of amino acids and ester groups. Generally, a more hydrophilic composition of the polymers exhibited the higher LCST. Such results indicate that the LCST of the polymers can be controlled by appropriate design of the polymers. These thermosensitive polymers were found to be hydrolytically degradable, and the series of these biodegradable thermosensitive polymers is expected to be useful for application to drug delivery systems. Details of the degradable properties of these thermosensitive polymers will be published separately.

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References and Notes

(1) Ding, Z.; Chen, G.; Hoffman, A. S. *Bioconjugate Chem.* **1996**, *7*, 121.
(2) Mukae, K.; Bae, Y. H.; Okano, T.; Kim, S. W. *Polym. J.* **1990**, *22*, 250.
(3) Taylor, L. D.; Cerankowski, L. D. *J. Polym. Sci.* **1975**, *13*, 2551.
(4) Katono, H.; Maruyama, A.; Ogata, N.; Sanui, K.; Okano, T.; Sakurai, Y. *J. Controlled Release* **1991**, *16*, 215.
(5) Okano, T.; Bae, Y. H.; Kim, S. W. *J. Controlled Release* **1990**, *11*, 255.
(6) Okano, T.; Yamada, N.; Okuhara, M.; Sakai, H.; Sakurai, Y. *Biomaterials* **1995**, *16*, 297.
(7) Monji, N.; Hoffman, A. S. *Appl. Biochem. Biotechnol.* **1987**, *14*, 107.
(8) Park, T. G.; Hoffman, A. S. *Appl. Biochem. Biotechnol.* **1988**, *19*, 1.
(9) Chen, G. H.; Hoffman, A. S. *Nature* **1995**, *373*, 49.
(10) Gutoska, A.; Bae, Y. H.; Jacobs, H.; Mohamad, F.; Mix, D.; Feijen, J.; Kim, S. W. *J. Biomed. Mater. Res.* **1995**, *29*, 811.
(11) Malstom, M.; Lindman, B. *Macromolecules* **1992**, *25*, 5440.
(12) Allcock, H. R.; Kuhacik, S. E.; Reed, C. S.; Napierala, M. E. *Macromolecules* **1996**, *29*, 3384.
(13) Allcock, H. R.; Dudley, G. K. *Macromolecules* **1996**, *29*, 1313.
(14) Allcock, H. R.; Napierala, M. E.; Vameron, C. G.; O'Connor, S. J. M. *Macromolecules* **1996**, *29*, 1951.
(15) Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W. *Nature* **1997**, *388*, 860.
(16) Greenstein, J. D.; Winitz, M. *Chemistry of the Amino Acids*; John Wiley and Sons: New York, 1961; pp 925−942.
(17) Sohn, Y. S.; Cho, Y. H.; Baek, H.-G.; Jung, O.-S. *Macromolecules* **1995**, *28*, 7566.
(18) Allcock, H. R.; Pucher, S. R.; Scopelianos, A. G. *Macromolecules* **1994**, *27*, 1071.
(19) Vandrope, J.; Schacht, E. *Polymer* **1996**, *37*, 3141.

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