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A Thermosensitive Poly(organophosphazene) Gel

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ABSTRACT: Thermosensitive poly(organophosphazenes) bearing α-amino-ω-methoxy-PEG (AMPEG) and hydrophobic L-isoleucine ethyl ester (IleOEt) as side groups have been synthesized, and their reversible sol−gel properties were investigated by means of ^{31}P NMR spectroscopy and viscometer. In an aqueous solution, the poly(organophosphazenes) exhibited four-phase transitions with temperature gradually increasing: a transparent sol, a transparent gel, a opaque gel, and a turbid sol. The gelation properties of the polymer were affected by several factors such as the composition of substituents, the chain length of AMPEG, and the concentration of the polymer solutions. The more hydrophilic composition of the polymers offers the higher gelation temperature. The gelation of the polymer is presumed to be attributed to the hydrophobic interaction between the side-chain fragments (−CH(CH$_3$)CH$_2$CH$_3$) of IleOEt which act as the physical junction in the polymer aqueous solution.

Introduction

Hydrogels usually have three-dimensional networks that are formed by covalent bonding or by physical association between polymer segments in aqueous solution. The cross-links of physical networks do not occur at points on the chain as do covalent cross-links but involve physical junction zones.[1,2] Thermoassociative polymers showing a reversible phase transition form physical gels owing to physical association between the polymer and solvent molecules or between polymer segments in an aqueous solution in response to temperature. In the case of neutral polymers, the physical network is usually considered to be formed by hydrophobic interaction between polymer chains such as alkyl, perfluoroalkyl, or aromatic fragments in aqueous solution. Hydrogels formed by physical association between polymer chains have a potential for biomedical applications because usually no toxic organic cross-linkers are employed. Copolymers of *N*-isopropylacrylamide,[3−6] modified polysaccharides,[7] and PEO−PPO−PEO block copolymers (Pluronics)[8] are known to form such hydrogels, but these polymers are nonbiodegradable, limiting their biomedical applications. In recent years biodegradable PEG−PLGA−PEG triblock copolymers with thermosensitive sol−gel properties were reported.[9−12] The biodegradable thermosensitive polymer hydrogels may be a preferable candidate as a protein delivery matrix because of its biosafety and inertness to protein drugs by avoiding exposure to heating, sonication, and organic solvents.

Polyphosphazene hydrogels were studied by the Tanigami group[13] and the Allcock group.[14,15] The hydrogel system developed by the Tanigami group was structurally too complicated to characterize. The hydrogels by the Allcock group were formed by covalent cross-links by means of γ-ray radiation and therefore were neither reversible to sols nor nonbiodegradable. We have recently reported biodegradable thermosensitive poly(organophosphazenes) and cyclotriphosphazenes bearing PEG and amino acid esters as side groups with a wide range of LCST (lower critical solution temperature).[16−18] These phosphazene derivatives were soluble in an aqueous solution below their LCST but precipitated above their LCST. More recently, we have found that polyphosphazenes with α-amino-ω-methoxy-PEG350 (AMPEG350) and hydrophobic L-isoleucine ethyl ester (IleOEt) as side groups exhibit reversible sol−gel transition in an aqueous solution depending on the solution temperature. Here we report synthesis and thermoassociative properties of these poly(organophosphazenes).

Experimental Section

Materials. Hexachlorocyclotriphosphazene (Aldrich) was used without further purification. L-Isoleucine ethyl ester (IleOEt) was prepared by the literature method.[19] α-Amino-ω-methoxy-poly(ethylene glycols) (AMPEGs) with molecular weights of 350 and 550 were prepared using the procedure by Loccufrier et al.,[20] followed by vacuum-drying, and then stored over molecular sieve 4 Å. Tetrahydrofuran (THF) was dried by reflux over sodium metal and distilled under a nitrogen atmosphere.

Synthesis of [NP(AMPEG350)$_{0.87}$(IleOEt)$_{1.13}$]$_n$ (1). Poly(dichlorophosphazene) was prepared as described previously.[21] L-Isoleucine ethyl ester hydrochloride (4.1 g, 20.7 mmol) suspended in dry THF (100 mL) containing triethylamine (8.4 g, 82.8 mmol) was added slowly to poly(dichlorophosphazene) (2.0 g, 17.3 mmol) dissolved in dry THF (100 mL). The reaction mixture was stirred for 4 h at 4 °C and then for 20 h at room temperature. After AMPEG350 (9.7 g, 27.6 mmol) dissolved in dry THF (100 mL) containing triethylamine (5.6 g, 55.2 mmol) was added to the polymer solution, the reaction mixture was stirred for 2 days at 40−50 °C. The reaction mixture was filtered. After the filtrate was concentrated, it was poured into *n*-hexane to obtain a precipitate, which was reprecipitated twice in the same solvent system. The polymer product was further purified by dialysis in methanol for 2 days and then in distilled water for 2 days at 4 °C. The final dialyzed solution was freeze-dried to obtain polymer **1**. Yield: 74%. ^{31}P NMR ($CDCl_3$), δ (ppm): 19.9. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.8−1.0 (s, 6H), 1.1−1.3 (b, 3H), 1.3−1.6 (b, 2H), 1.6−1.9 (b, 1H), 2.8−3.1 (b, 2H), 3.4 (s, 3H), 3.5−3.9 (b, 26H), 3.9−4.1(b, 1H), 4.1−4.3 (b, 2H). Elem Anal. (%) Calcd: C, 50.40; H, 8.79; N, 7.98. Found: C, 49.80; H, 8.84; N, 8.24. T_g = −67 °C.

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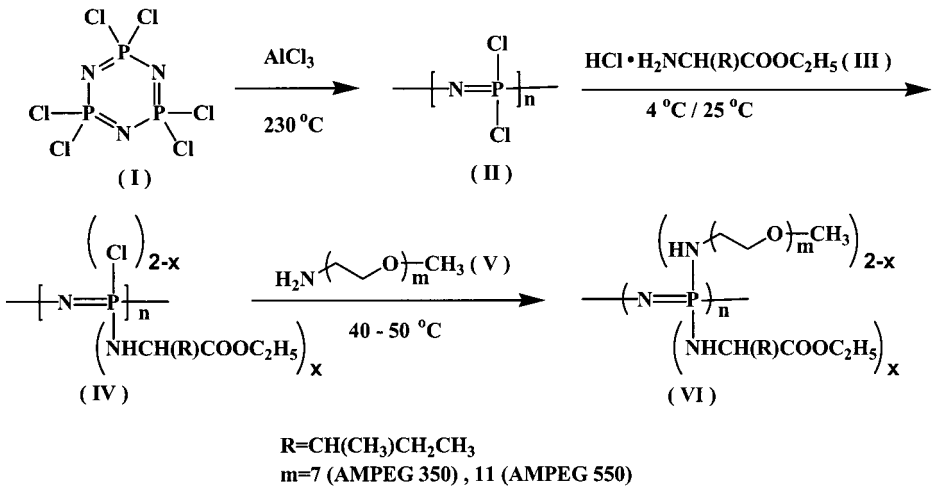
Scheme 1



R=CH(CH$_3$)CH$_2$CH$_3$
m=7 (AMPEG 350) , 11 (AMPEG 550)

The other polymers were prepared analogously using different mole ratios and molecular weights of AMPEG.

[NP(AMPEG350)$_{1.06}$(IleOEt)$_{0.94}$]$_n$ (2). IleOEt (19.0 mmol) and AMPEG350 (31.0 mmol). Yield: 56%. ^{31}P NMR ($CDCl_3$), δ (ppm): 19.1. Elem Anal. (%) Calcd: C, 50.01; H, 8.77; N, 7.47. Found: C, 49.80; H, 8.80; N, 7.67. $T_g = -69$ °C.

[NP(AMPEG350)$_{1.45}$(IleOEt)$_{0.55}$]$_n$ (3). IleOEt (10.4 mmol) and AMPEG350 (48.3 mmol). Yield: 70%. ^{31}P NMR ($CDCl_3$), δ (ppm): 19.7. Elem Anal. (%) Calcd: C, 49.34; H, 8.74; N, 6.60. Found: C, 49.70; H, 8.98; N, 6.78. $T_g = -70$ °C.

[NP(AMPEG550)$_{0.76}$(IleOEt)$_{1.24}$]$_n$ (4). IleOEt (20.7 mmol) and AMPEG550 (27.6 mmol). Yield: 65%. ^{31}P NMR ($CDCl_3$), δ (ppm): 19.0. Elem Anal. (%) Calcd: C, 51.44; H, 8.98; N, 6.39. Found: C, 51.10; H, 9.00; N, 6.57. $T_g = -67$ °C.

Instruments and Measurements. Elemental analysis was carried out with a Fisons 1108 CHNS Microanalizer 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. A higher resolution NMR spectrometer (Varian UI-500) was used for ^{31}P NMR studies on the phase transition behaviors in the range 5−60 °C. Thermal analysis of the polymers was carried out using a Dupont DSC 2100 TA Instruments. The sample was heated at a rate of 5 °C/min in the range of −100 to 100 °C. Gel permeation chromatography was carried out using a GPC system (Waters 1515) with a refractive index detector (Waters 2410) and two styragel columns (Waters styragel HR 5E) connected in line at a flow rate of 0.8 mL/min at 35 °C. Polystyrenes (M_w = 1140, 3570, 14 100, 28 700, 65 300, 181 000, 613 000, 1 010 000, and 2 660 000) were used as standards to calibrate the column. The viscosity of the aqueous solutions of polymers (5, 7.5, and 10 wt %) was measured as a function of temperature: viscosity measurements on polymer solutions were carried out on a Brookfield RVDV-III+ viscometer between 5 and 60 °C with a slow heating rate of 0.04 °C/min to preclude any kinetic effect and under the shear rate of 1.7 s^{-1}. A cooling process followed the heating process in the same conditions. The phase transition of the polymer aqueous solutions (10 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.

Results and Discussion

The present polymers were prepared by the synthetic Scheme 1. Poly(dichlorophosphazene) (**II**) dissolved in THF was allowed to react with IleOEt (**III**) to yield the partially substituted polymer (**IV**), which was then allowed to react with α-amino-ω-methoxy-PEG350 (**V**) to obtain the final polymer products (**VI**). Different copolymers (**1**−**4**) were obtained by variation of the mole ratio of the two substituents. The polymer products obtained were characterized by means of multinuclear NMR spectroscopies, DSC, GPC, and elemental analysis. The stepwise substitution reactions were monitored by ^{31}P NMR spectroscopy, and the ^{31}P NMR spectral change during the synthetic process for polymer **1** is shown in Figure 1. When poly(dichlorophosphazene) (**II**) was allowed to react with IleOEt (**III**), the intermediate (**IV**) showed major peaks at 19.9 and 10.5 ppm with some side peaks. After the intermediate (**IV**) was allowed to react with AMPEG350 (**V**), one major peak at 10.5 ppm as well as the side peaks disappeared, finally giving a major peak at 19.9 ppm, which is assigned to the phosphorus resonance of the final copolymer (**VI**). It may be presumed from ^{31}P NMR spectra that chlorine atoms were completely replaced by subsequent reaction with α-amino-ω-methoxy-PEG350. All of the polymers substituted by IleOEt and AMPEG were obtained as

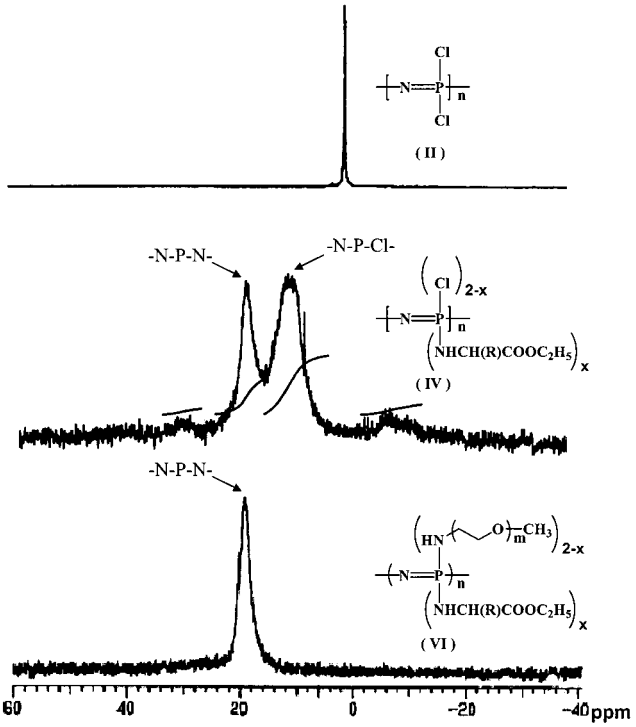


Figure 1. ^{31}P NMR spectral change monitored during the substitution reaction for polymer **1**.

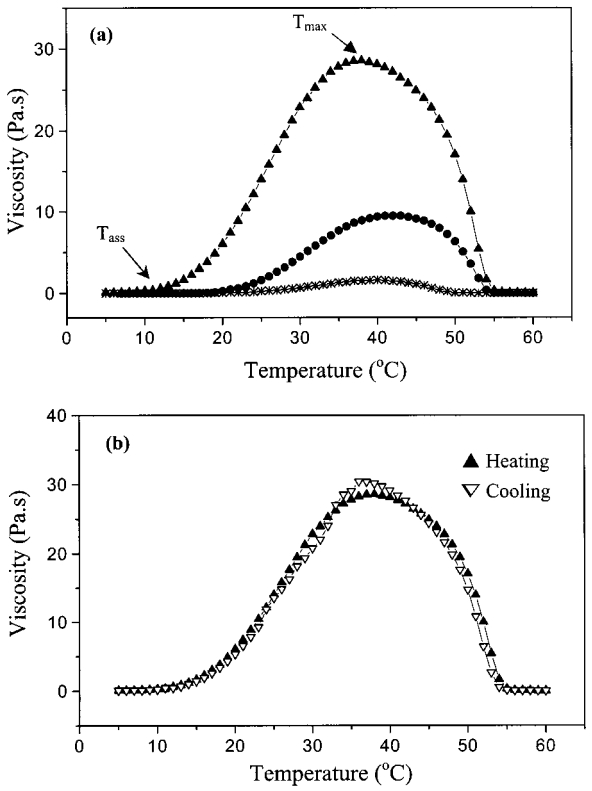


Figure 2. (a) Viscosity change of 5 (∗), 7.5 (●), and 10 wt % (▲) aqueous solutions of polymer **2** as a function of temperature under shear rate 1.7 s^{-1}. (b) Viscosity change of the aqueous solution of polymer **2** (10 wt %) as a function of temperature by heating (▲) and by cooling (▽) under shear rate 1.7 s^{-1}.

pale yellow viscoelastic solids, which were soluble in cold water and in several organic solvents such as chloroform, tetrahydrofuran, and methyl alcohol.

The gelation behavior of polymer **2** in aqueous solutions (5−10 wt %) examined by measuring the viscosity as a function of temperature is shown in Figure 2a. The clear polymer solution (10 wt %) at low temperature starts to become viscous as temperature is raised to about 11 °C (T_{ass}), and its viscosity reaches the maximum at 38 °C (T_{max}). The gel formed at 38 °C is transparent but becomes gradually opaque as the temperature is further raised from 38 °C and then gradually starts to shrink by expelling water, leading to a shrunken gel. Beyond this temperature, its viscosity grows lower with increasing temperature, and finally, a turbid solution is obtained at around 55 °C. When this turbid solution was cooled in the same conditions, the viscosity vs temperature curve followed almost the same pattern as the curve on heating, as shown in Figure 2b. The T_{max} values of polymer **2** on heating and on cooling are 38 and 36 °C, respectively, indicating that no important hysteresis exists. It has been reported that the gelation of the thermosensitive polymers occurs via a physical cross-linking mechanism or a micellar aggregation mechanism, and the mechanism is dependent on the polymer structure.[22] The gelation of the present polymer is presumed to be attributed to the hydrophobic interaction between the side-chain fragments (−$CH(CH_3)$-CH_2CH_3) of IleOEt which act as the physical junction in the polymer aqueous solution. In particular, at around T_{max} such a physical cross-linking seems to be retained while strong hydrogen-bonding interaction between the hydrophilic parts of the polymer and water molecules is preserved, affording a transparent gel. But a further increase in the polymer solution temperature beyond T_{max} may cause the hydrogen-bonding interaction to be broken, resulting in the formation of a shrunken gel following an opaque gel. We examined the micellar behavior of the present polymers by fluorescence and light scattering measurements, but the micellar behavior was not observed in a wide range of temperature and concentration of the aqueous polymer solution. This result is consistent with the aforementioned physical cross-linking mechanism of the present polymer. The gelation behavior of the polymers was dependent on its concentration. The sharpness of the thermothickening curves and the magnitude of their viscosity increased with increasing concentration of polymer **2** solutions: The viscosity of 5 wt % solution only slightly increased above 29 °C while the viscosities of 7.5 and 10 wt % solutions increased more steeply at 20 and 11 °C, respectively.

As shown in Table 1, the T_{ass}, T_{max}, and T_{lcst} values of the polymer solutions increase with decreasing content of IleOEt: The T_{ass}, T_{max}, and T_{lcst} values (7, 29, and 37 °C, respectively) for polymer **1** with 1.13 mol of IleOEt increased to higher values of 11, 38, and 55 °C, respectively, for polymer **2** with 0.94 mol of IleOEt. Polymer **3** with the lower content of IleOEt (0.55 mol) showed a higher T_{lcst} value (74 °C) than polymers **1** and **2** but did not have T_{ass} and T_{max} probably due to both low content of IleOEt and its high hydrophilicity. The longer chain of AMPEG gave higher T_{ass}, T_{max}, and T_{lcst} values as demonstrated by polymers **1** and **4** showing their T_{ass} values of 7 and 35 °C, T_{max} values of 29 and 61 °C, and T_{lcst} values of 37 and 75 °C, respectively. From such results, it could be inferred that increased hydrophilicity of the polymer increases T_{ass}, T_{max}, and T_{lcst} values of the polymer solution, and the magnitude of the thickening process can be evaluated by the V_{max} values of the polymer solutions. For polymers **1**, **2**, and **3** with the same side chains but different mole ratios, the higher content of IleOEt gave rise to the higher V_{max} value: The V_{max} values for polymers **1** and **2** were 116.9 and 28.6 Pa·s, respectively, but polymer **3** had no thermothickening property owing to the low content of IleOEt which was presumed to act as a moiety of physical junction.

Figure 3 shows temperature-dependent ^{31}P NMR spectra of polymer **2** in aqueous solution (7.5 wt %) along

Table 1. Characteristics of Poly(organophosphazenes)

polymer	structure	T_{ass} (°C)[a]	T_{max} (°C)[b]	T_{lcst} (°C)[c]	V_{max} (Pa·s)[d]	MW[e]
1	$[NP(AMPEG350)_{0.87}(IleOEt)_{1.13}]_n$	7	29	37	116.9	3.9×10^4
2	$[NP(AMPEG350)_{1.06}(IleOEt)_{0.94}]_n$	11	38	55	28.6	2.1×10^4
3	$[NP(AMPEG350)_{1.45}(IleOEt)_{0.55}]_n$			74		6.0×10^4
4	$[NP(AMPEG550)_{0.76}(IleOEt)_{1.24}]_n$	35	61	75	5.0	2.0×10^4

[a] The association temperature at which the viscosity of the polymer solutions (10 wt %) begins to increase sharply. [b] The temperature at which the polymer solutions (10 wt %) reach their maximum viscosity. [c] The LCST was identified as the temperature at which the polymer solutions (10 wt %) became turbid. [d] The viscosity of the polymer solutions at T_{max}. [e] The molecular weight of the polymers was measured by GPC using THF solutions containing 0.1% (w/v) TBAB (tetrabutylammonium bromide).

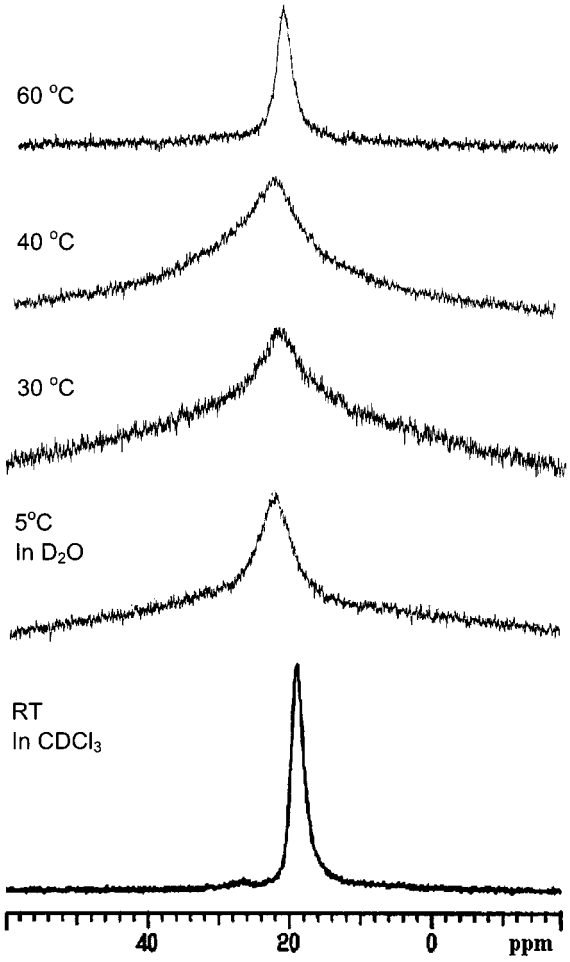


Figure 3. Temperature-dependent ^{31}P NMR spectra of polymer **2** in D_2O (7.5 wt %) along with a ^{31}P NMR spectrum in $CDCl_3$ at room temperature.

with its room temperature spectrum in $CDCl_3$. The polymer is expected to have amphiphilicity because it bears both the hydrophilic AMPEG350 and the hydrophobic IleOEt as side groups on the polymer backbone. The ^{31}P NMR spectrum of polymer **2** in $CDCl_3$ showed a sharp peak at 19.5 ppm at room temperature, while a broad peak at 22.1 ppm was observed in D_2O at 5 °C. This result implies that the polymer molecules may move freely in chloroform at room temperature while they have some restriction in molecular motion in water at low temperature. As the temperature increased from 5 to 40 °C in D_2O, the phosphorus resonance peak became further broadened, indicating that the polymer solution became a gel at the higher temperature. However, a further increase in the temperature of the polymer solution to 60 °C gave rise to a sharper phosphorus resonance peak than the peak at 5 °C. It may be assumed from such a result that the gel structure is broken to become a turbid sol and a small amount of the polymer dissolves in water at the high temperature, resulting in the appearance of the sharp phosphorus peak. The ^1H NMR spectra of polymer **2** also have shown exactly the same temperature-dependent behavior. Similar phenomena were observed in other thermosensitive polymers.[10,23,24]

In conclusion, thermosensitive poly(organophosphazene) gels bearing AMPEG and IleOEt have been synthesized, and their reversible sol−gel properties were investigated. The poly(organophosphazenes) synthesized showed gelation behavior from a transparent gel to a shrunken gel with increasing temperature of the aqueous polymer solutions. The gelation properties of the present polymers were affected by the composition of substituents, the chain length of AMPEG, and the concentration of the polymer solutions. These polymer gels are biodegradable and useful for application to injectable drug delivery systems.[25−28] Further details will be published separately.

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