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New thermogelling poly(organophosphazenes) with methoxypoly(ethylene glycol) and oligopeptide as side groups

Ji-Yeon Seong[a], Yong Joo Jun[a,b], Byeongmoon Jeong[a], Youn Soo Sohn[a,*]

[a]*Division of Nano Science, Department of Chemistry, Ewha Womans University, Daehyun-Dong 11-1, Seodaemun-Ku, Seoul 120-750, South Korea*
[b]*Department of Chemistry, Yonsei University, Seoul 120-749, South Korea*

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Abstract

A new class of thermogelling poly(organophosphazenes) bearing a hydrophilic methoxypoly(ethylene glycol) (MPEG) and a hydrophobic tri or tetrapeptide such as GlyPheLeuEt, GlyPheIleEt, GlyLeuPheEt, and GlyPheLeuGlyEt have been synthesized and characterized by means of multinuclear (^{1}H, ^{31}P) NMR spectroscopy, gel permeation chromatography, viscometry, and elemental analysis. The gelation of the present polymers is presumed to be attributed to the intermolecular interaction between the hydrophobic oligopeptide side groups, which can form strong physical junction zones in the polymer aqueous solution. The gelation properties of the polymer were affected by the subtle change in the nature of the hydrophobic oligopeptide, composition of the hydrophilic to hydrophobic side groups, and the concentration of the polymer solutions. Among the present thermogels, the copolymer with equimolar MPEG and GlyPheIleEt as side groups showed the excellent gel phase persisting over 35–43 °C, which indicates that it is a new promising material for drug delivery and tissue engineering.

Keywords: Polyphosphazene; Gel; Oligopeptide

1. Introduction

Polymer gels have drawn a great deal of attention in recent years because of their potential applications in numerous high-tech fields such as drug delivery systems, permeable membranes, sensor devices, and other biomedical applications [1]. Polymer gels usually have three-dimensional networks that are formed by covalent bonding or by physical association between polymer segments in aqueous solution. Chemical gels are cross-linked by covalent bonds and thus their sol–gel transition is irreversible, whereas physical gels are cross-linked by weak forces such as hydrogen bonding and/or hydrophobic interactions forming physical junction zones [2,3], and their sol–gel transition is reversible. Thermoassociative polymers showing a reversible phase transition form physical gels owing to physical association between the hydrophobic polymer segments in 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. The polymer gels formed by physical association between polymer chains include copolymers of *N*-isopropylacrylamide [4–7], modified polysaccharides [8], PEO–PPO–PEO block copolymers (Pluronics) [9], and PEG–PLGA–PEG triblock copolymers [10–13]. However, most of these polymer gels need further improvements for delivery of sensitive bio-drugs such as protein and DNA drugs because of non-biodegradability or acidic degradation of the polymers. Therefore, there is an urgent demand for more biocompatible thermosensitive hydrogels which are suitable for delivery of such biodrugs.

We have recently reported biodegradable thermosensitive poly(organophosphazenes) and cyclotriphosphazenes bearing PEG and amino acid esters as side groups with a wide range of lower critical solution temperature (LCST) [14–16]. More recently, we have also reported the first physical gel of poly(organophosphazene) synthesized using a hydrophilic α-amino-ω-methoxy-PEG (AMPEG) and a hydrophobic L-isoleucine ethyl ester (IleOEt) as side groups [17]. This hydrogel exhibited reversible sol–gel transition in

* Corresponding author. Tel.: +82 2 3277 2345; fax: +82 2 3277 2384.
E-mail address: yssohn@ewha.ac.kr (Y.S. Sohn).

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aqueous solution, but was found to be not strong enough for practical applications as drug delivery systems. As a matter of fact, all of our earlier attempts to prepare polymer gels using the combination of PEGs and amino acids as hydrophilic and hydrophobic side groups, respectively, were not successful, probably because the amino acids were not able to form strong physical junction zones enough to give a gel with PEG. Instead, we could make a gel only from the combination of AMPEG and IleOEt above-mentioned, but no other amino acids gave rise to a gel with neither AMPEG nor PEG.

However, we have found in this study that by employing oligopeptides such as more hydrophobic and longer chain tri or tetrapeptides than amino acids along with PEG we could design and synthesize a new class of thermogelling poly(organophosphazenes) with a variety of thermal properties depending on the molecular structure of the oligopeptides but also with higher gel strength compared to our previous gel bearing AMPEG and amino acid as side groups [17]. Here, we report synthesis, characterization, and properties of these new poly(organophosphazene) gels.

2. Experimental

2.1. Materials

Hexachlorocyclotriphosphazene (Aldrich) was used without further purification. The tripeptides, glycyl-L-phenylalanyl-L-leucine ethyl ester (GlyPheLeuEt), glycyl-L-phenylalanyl-L-isoleucine ethyl ester (GlyPheIleEt), glycyl-L-leucyl-L-phenylalanine ethyl ester (GlyLeuPheEt), and the tetrapeptide, glycyl-L-phenylalanyl-L-leucyl-glycyl ethyl ester (GlyPheLeuGlyEt) were prepared by the literature methods [18]. Methoxy poly(ethylene glycol) with molecular weight of 350 (MPEG350) (Fluka) was used without further purification but thoroughly vacuum dried and then stored over molecular sieve 4 Å before use. Tetrahydrofuran (THF) was dried by boiling at reflux over sodium metal and benzophenone, and then distilled under a nitrogen atmosphere. Chloroform and triethylamine were dried by boiling at reflux over sodium hydride and barium oxide, respectively, and then distilled under the same condition.

2.2. Instruments and measurements

Elemental analysis was carried out with a Carlo Erba-EA1108. ^{1}H NMR measurements were made with a Varian Gemini-250 spectrometer operating at 250 MHz in the Fourier transform mode. Proton-decoupled ^{31}P NMR spectra were measured with a Varian Unity INOVA-400 spectrometer operating at 400 MHz using phosphoric acid as an external standard. A higher resolution NMR spectrometer (Bruker Avance 500) was used for ^{1}H NMR studies on the phase transition behaviors in the range 5–60 °C. Gel permeation chromatography was carried out using a Waters Associates HPLC/GPC 150C unit and two styragel columns (Waters styragel HT 4) connected in line at a flow rate of 1.0 ml/min at 40 °C and fitted with a refraction index detector and a computerized data station. THF was used as an eluting solvent. Poly(ethylene oxide) (M_w = 6000, 11,200, 24,800, 42,900, 149,000, 348,000, 722,000, and 531,000) was used as standard. The viscosity of the aqueous solutions of polymers (5, 10, and 15 wt%) was measured as a function of temperature: viscosity measurements of polymer solutions were carried out on a Haake RheoStress 1 viscometer between 5 and 60 °C with a slow heating rate of 0.2 °C/min to preclude any kinetic effect and under the shear rate of 1.7 s^{-1}. 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.

2.3. Synthesis

2.3.1. $[NP(MPEG350)_{1.0}(GlyPheLeuEt)_{1.0}]_n$(**1**)

Poly(dichlorophosphazene) was prepared as described previously [19]. The sodium salt of methoxy-poly(ethylene glycol) (MPEG350) was prepared by reaction of MPEG350 (3.17 g, 9.06 mmol) with 1.05 equivalent of sodium hydride in THF (150 ml) at room temperature for 5 h. The solution was dropped slowly to poly(dichlorophosphazene) (1.0 g, 8.63 mmol) dissolved in THF (80 ml). The reaction mixture was stirred for 12 h at −78 °C. Meanwhile, glycyl-L-phenylalanyl-L-leucine ethyl ester (3.77 g, 10.36 mmol) was dissolved in dry chloroform (100 ml) containing three equivalent of dry triethylamine. The glycyl-L-phenylalanyl-L-leucine ethyl ester solution was added to the polymer solution, which was stirred for 2 days at 50 °C. The reaction mixture was filtered to remove triethylammonium chloride precipitated. After the filtrate was evaporated, the concentrate was precipitated using a solvent pair of THF and *n*-hexane to obtain a yellow precipitate, which was repeated twice in the same solvent system. In order to remove unreacted molecules and inorganic salt, the solution was dialyzed for 1 day against methanol and another day against ultrapure water using cellulose dialysis membrane (M_w cutoff: 3.5×10^3, Spectrum Co). The dialyzed solution was freeze-dried to obtain polymer **1**. Other polymers were prepared analogously using different oligopeptides and mole ratios of the two side groups.

Yield: 50%. ^{31}P NMR ($CDCl_3$), δ (ppm): 0.72. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.7–1.0 (b, 5.8H), 1.1–1.4 (b, 3.9H), 1.4–1.8 (b, 2.6H), 2.9–3.2 (b, 1.6H), 3.2–3.3 (s, 3.0H), 3.5–3.9 (b, 23.3H), 3.9–4.3 (b, 4.7H), 4.3–4.7 (b, 1.7H), 7.0–7.4 (b, 5.2H) Elem Anal. (%) Calcd for $[NP(C_{15}H_{31}O_8)_{1.0}(C_{19}H_{28}O_4N_3)_{1.0}\cdot 2H_2O]$: C, 52.16; H, 8.11; N, 7.16. Found: C, 52.31; H, 8.71; N, 7.42.

*2.3.2. $[NP(MPEG350)_{1.0}(GlyPheIleEt)_{1.0}]_n$ (**2**)*

MPEG350 (9.06 mmol) and GlyPheIleEt (10.36 mmol) were used. Yield: 57%. ^{31}P NMR ($CDCl_3$), δ (ppm): −0.31. ^1H NMR ($CDCl_3$), δ (ppm): 0.4–1.0 (b, 4.4H), 1.0–1.2 (b, 3.3H), 1.2–1.4 (b, 0.7H), 1.6–1.9 (b, 0.8H), 2.9–3.1 (b, 4.8H), 3.2–3.3 (s, 3.0H), 3.3–3.7 (b, 19.0H), 3.7–4.2 (b, 3.0H), 4.2–4.5 (b, 0.8H), 4.5–4.7 (b, 0.5H), 6.8–7.2 (b, 3.5H). Elem Anal. (%) Calcd for $[NP(C_{15}H_{31}O_8)_{1.0}(C_{19}H_{28}O_4N_3)_{1.0}\cdot 2H_2O]$: C, 52.16; H, 8.11; N, 7.16. Found: C, 52.19; H, 8.16; N, 7.67.

*2.3.3. $[NP(MPEG350)_{0.8}(GlyPheIleEt)_{1.2}]_n$ (**3**)*

MPEG350 (7.25 mmol) and GlyPheIleEt (12.43 mmol) were used. Yield: 61%. ^{31}P NMR ($CDCl_3$), δ (ppm): 0.95. ^1H NMR ($CDCl_3$), δ (ppm): 0.3–1.0 (b, 4.0H), 1.0–1.3 (b, 2.7H), 1.3–1.5 (b, 0.6H), 1.5–2.0 (b, 0.9H), 2.8–3.2 (b, 1.4H), 3.2–3.3 (s, 3.0H), 3.3–3.8 (b, 13.9H), 3.8–4.3 (b, 2.8H), 4.3–4.6 (b, 1.0H), 6.8–7.4 (b, 3.3H). Elem Anal. (%) Calcd for $[NP(C_{15}H_{31}O_8)_{0.8}(C_{19}H_{28}O_4N_3)_{1.2}\cdot 3H_2O]$: C, 51.96; H, 8.07; N, 8.01. Found: C, 51.95; H, 7.58; N, 7.96.

*2.3.4. $[NP(MPEG350)_{1.1}(GlyPheIleEt)_{0.9}]_n$ (**4**)*

MPEG350 (9.97 mmol) and GlyPheIleEt (9.32 mmol) were used. Yield: 43%. ^{31}P NMR ($CDCl_3$), δ (ppm): 0.426, −3.083. ^1H NMR ($CDCl_3$), δ (ppm): 0.3–0.7 (b, 3.8H), 0.7–1.1 (b, 2.8H), 1.1–1.3 (b, 0.63H), 1.4–1.7 (b, 0.75H), 2.6–3.0 (b, 1.05H), 3.0–3.1 (s, 3.0H), 3.1–3.5 (b, 23.1H), 3.5–3.7 (b, 0.85H), 3.7–4.0 (b, 2.95H), 4.0–4.3 (b, 0.95H), 6.7–7.1 (b, 3.4H). Elem Anal. (%) Calcd for $[NP(C_{15}H_{31}O_8)_{1.1}(C_{19}H_{28}O_4N_3)_{0.9}\cdot 2H_2O]$: C, 51.67; H, 8.17; N, 6.64. Found: C, 52.24; H, 8.04; N, 6.87.

*2.3.5. $[NP(MPEG350)_{0.8}(GlyLeuPheEt)_{1.2}]_n$ (**5**)*

MPEG350 (8.15 mmol) and GlyLeuPheEt (12.43 mmol) were used. Yield: 43%. ^{31}P NMR (DMSO), δ (ppm): 4.97. ^1H NMR (DMSO), δ (ppm): 0.7–1.0 (b, 3.8H), 1.0–1.3 (b, 3.6H), 1.3–1.8 (b, 1.6H), 2.8–3.1 (b, 1.1H), 3.1–3.2 (s, 3.0H), 3.2–3.8 (b, 23.0H), 3.8–4.3 (b, 3.9H), 4.3–4.8 (b, 1.7H), 7.1–7.4 (b, 4.2H). Elem Anal. (%) Calcd for $[NP(C_{15}H_{31}O_8)_{0.8}(C_{19}H_{28}O_4N_3)_{1.2}\cdot 2H_2O]$: C, 53.15; H, 7.99; N, 8.19. Found: C, 52.93; H, 7.52; N, 8.14.

*2.3.6. $[NP(MPEG350)_{0.7}(GlyLeuPheEt)_{1.3}]_n$ (**6**)*

MPEG350 (6.34 mmol) and GlyLeuPheEt (13.46 mmol) were used. Yield: 74%. ^{31}P NMR ($CDCl_3$), δ (ppm): 0.93. ^1H NMR (DMSO), δ (ppm): 0.7–1.1 (b, 5.2H), 1.1–1.4 (b, 4.3H), 1.4–1.8 (b, 3.2H), 2.9–3.2 (b, 1.8H), 3.2–3.3 (s, 3.0H), 3.3–4.0 (b, 25.8H), 4.0–4.4 (b, 6.2H), 4.4–4.7 (b, 3.7H), 7.1–7.4 (b, 5.0H). Elem Anal. (%) Calcd for $[NP(C_{15}H_{31}O_8)_{0.7}(C_{19}H_{28}O_4N_3)_{1.3}\cdot 3H_2O]$: C, 52.34; H, 7.99; N, 8.50. Found: C, 51.93; H, 7.52; N, 8.14.

*2.3.7. $[NP(MPEG350)_{1.0}(GlyPheLeuGlyEt)_{1.0}]_n$ (**7**)*

MPEG350 (9.06 mmol) and GlyPheLeuGlyEt (10.36 mmol) were used.Yield: 53%. ^{31}P NMR ($CDCl_3$), δ (ppm): 2.709. ^1H NMR ($CDCl_3$), δ (ppm): 0.5–1.1 (b, 4.9H), 1.1–1.4 (b, 3.4H), 1.4–1.6 (b, 1.9H), 2.8–3.2 (b, 1.2H), 3.3–3.4 (s, 3.0H), 3.4–3.8 (b, 20.8H), 3.8–4.3 (b, 5.3H), 6.8–7.5 (b, 5.0H). Elem Anal. (%) Calcd for $[NP(C_{15}H_{31}O_8)_{1.0}(C_{21}H_{31}O_5N_4)_{1.0}\cdot H_2O]$: C, 52.61; H, 7.85; N, 8.52. Found: C, 52.32; H, 7.92; N, 9.06.

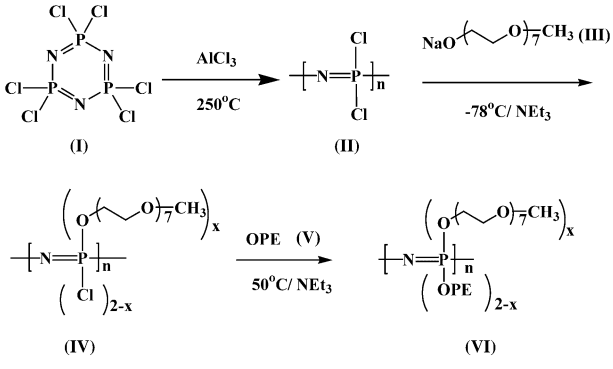
3. Results and discussion

3.1. Synthesis and characterization

The present poly(organophosphazenes) were prepared by the synthetic Scheme 1. Poly(dichlorophosphazene) (**II**) dissolved in THF was allowed to react with MPEG350 (**III**) to yield the partially substituted polymer (**IV**), which was then reacted with tri or tetrapeptide (**V**) to obtain the final polymer products (**VI**). Different copolymers (**1**–**7**) were obtained by variation of the oligopeptide structure and the mole ratio of the two substituents. The polymer products obtained were characterized by means of multinuclear NMR spectroscopies, GPC, viscometry, and elemental analysis.

The stepwise nucleophilic substitution reactions of the chloropolymers (**II**) with MPEG350 (**III**) and the oligopeptide (**V**) were monitored by ^{31}P NMR spectroscopy. The typical spectral change during the synthetic process for polymer **1** is shown in Fig. 1.

When poly(dichlorophosphazene) (**II**) was allowed to react with MPEG350 (**III**), the partially substituted intermediate (**IV**) showed a major peak at −11.80 and a side peak at −20.91 ppm. After the intermediate (**IV**) was reacted with tri or tetrapeptide (**V**), these peaks disappeared, finally giving a broad major peak at −0.31 ppm, which is assigned to the phosphorus resonance of the final copolymer (**VI**). It is presumed from ^{31}P NMR spectra that chlorine atoms were completely replaced by subsequent reaction with tri or tetrapeptide. The phosphorus resonance peak of the final product is broad probably owning to the restricted degree of freedom of the tri, or tetrapeptide side group. All the final polymer products were obtained as pale yellow viscoelastic solids, which were soluble in cold water and in several organic solvents such as chloroform, tetrahydrofuran, and methyl alcohol. The copolymers thus obtained in



Scheme 1. Synthetic route to poly(organophosphazene) gels.

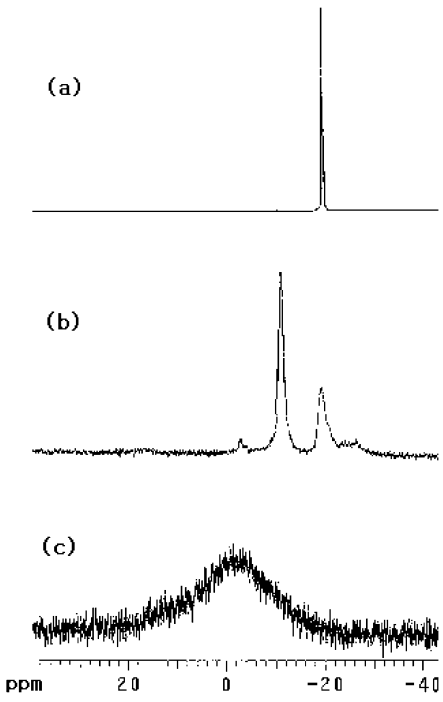


Fig. 1. ^{31}P NMR spectral change monitored during the substitution reactions for polymer **1**: (a) poly(dichlorophosphazene) (**II**), (b) the partially substituted polymer (**IV**) and (c) the final copolymer product (**VI**).

this study are listed along with their characteristic properties in Table 1. The data for the thermal properties and gel viscosities in the table were measured for 10% polymer solutions, but polymer **4** bearing a low content of the tripeptide did not form a gel even at higher concentrations.

In our previous work [17], we have attempted to prepare a poly(organophosphazene) gel using variable combinations of MPEG or AMPEG as a hydrophilic side group and various amino acids as a hydrophobic side group. However, we could make a gel only from the combination of isoleucine ethyl ester (IleOEt) among amino acids and α-amino-ω-methoxy-PEG (AMPEG) containing a terminal amine group, which can afford stronger hydrogen bonding to hold the solvent water molecules in the gel. We could not find any other combination resulting in a gel. However, we have found in the present study that by employing oligopeptides we could design various thermogels with different thermal properties depending on the molecular structure and relative composition of the oligopeptides. Furthermore, in contrast to our previous gel (V_{max} = 28.6 Pa s) prepared from AMPEG and IleOEt, the present gels seem to be strong enough and suitable for local delivery of peptide and protein drugs.

3.2. Gelation behavior

As is seen in Table 1, both gelation temperature (T_{max}) and maximum gel viscosity (V_{max}) of the present copolymers are variable in a wide range depending on the molecular structure and mole fraction of the hydrophobic oligopeptide side group. Most of the present copolymers form thermogels near body temperature at least in more than 10% aqueous solution, but polymer **7** exhibits much higher gelation temperature at 58 °C, probably because the tetrapeptide, GlyPheLeuGlyEt (log k = 0.03), is much less hydrophobic than the tripeptide, GlyPheLeuEt (log k = 1.11) [20]. It should also be noted that polymer **4** bearing the low mole fraction of the hydrophobic tripeptide do not form a gel, implying that the hydrophilic to hydrophobic balance is a critical factor for gel formation.

The gelation behavior of polymer **1** in aqueous solution (5–20 wt%) was examined by measuring the viscosity as a function of temperature and shown in Fig. 2(a). As shown in the figure, polymer **1** does not form a gel in 5% aqueous solution, but a strong gel is formed in more than 10% solution. The maximum viscosity (V_{max}) of polymer **1** is strongly dependent on its concentration, but its gelation temperature (T_{max}) at 25 °C is not largely dependent on its concentration. However, the critical disadvantage of this gel for local drug delivery is that the gel is rapidly collapsed beyond T_{max} before body temperature. On the other hand, polymer **2** in Fig. 2(b) not only exhibits the gelation temperature (T_{max} = 37 °C) at body temperature, but also forms a stable and strong gel over 35–43 °C, indicating that

Table 1
Characteristic properties of poly(organophosphazenes)

Polymer	Structure	T_{ass} (°C)[a]	T_{max} (°C)[b]	T_{lcst} (°C)[c]	V_{max} (Pa s)[d]	M_w[e]
1	$[NP(MPEG350)_{1.0}(GlyPheLeuEt)_{1.0}]_n$	15	25	30	56.1	6.7×10^4
2	$[NP(MPEG350)_{1.0}(GlyPheIleEt)_{1.0}]_n$	32	37	44	73.5	15×10^4
3	$[NP(MPEG350)_{0.8}(GlyPheIleEt)_{1.2}]_n$	12	28	39	330	16×10^4
4	$[NP(MPEG350)_{1.1}(GlyPheIleEt)_{0.9}]_n$	–	–	50	–	12×10^4
5	$[NP(MPEG350)_{0.8}(GlyLeuPheEt)_{1.2}]_n$	27	32	35	53.3	18×10^4
6	$[NP(MPEG350)_{0.7}(GlyLeuPheEt)_{1.3}]_n$	15	30	42	426	15×10^4
7	$[NP(MPEG350)_{1.0}(GlyPheLeuGlyEt)_{1.0}]$	35	58	70	3047	15×10^4

[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).

it is a promising biomaterial for drug delivery and tissue engineering applications.

Therefore, the phase changes of polymer **2** depending on the temperature were closely examined. The clear polymer solution (10 wt%) at low temperature starts to become viscous as temperature is raised to about 32 °C (T_{ass}), and its viscosity reaches the maximum (V_{max} = 73.5 Pa s) at 37 °C (T_{max}). The gel formed at 37 °C is transparent but becomes gradually opaque as the temperature is further raised beyond 37 °C and then starts to shrink by expelling water, leading to a shrunken gel. Beyond this temperature, its viscosity gradually decreases with increasing temperature, and finally, a turbid solution is obtained at around 44 °C. Such phase changes could also be confirmed by NMR spectroscopy.

Fig. 3 shows temperature-dependent ^{31}P NMR spectra of polymer **2** in D_2O (7.5 wt%). As the temperature increased from 5 to 40 °C, the peak is more broadened, indicating that the polymer solution become a gel in this temperature range, which is in accordance with the above observation. However, further increase in the temperature of the polymer solution to 60 °C results in a less broadened peak with asymmetry, probably due to the broken gel structure to turbid sol and some amount of the polymer dissolved in water at the high temperature. The ^{1}H NMR spectra of the polymer **2** solution in Fig. 4 also have shown a similar temperature-dependent behavior. Especially, among the tripeptide resonances, the proton peaks of $-C_6H_5$ (6.8–7.2 ppm) in PheEt and of ($-CH(CH_3)CH_2CH_3$) (0.4–1.9 ppm) in IleEt are nearly not observable under the gelation temperature, indicating that the micellar aggregation mechanism is involved, but at higher temperatures these peaks clearly appear probably due to the destruction of the physical junctions formed by the hydrophobic oligopeptide segments. Similar phenomena were observed in other thermosensitive polymers [11,21,22]. We believe that such a profile of viscosity vs. temperature of polymer **2** seems to be an ideal system for local delivery of hydrophobic drugs such as protein and peptide drugs.

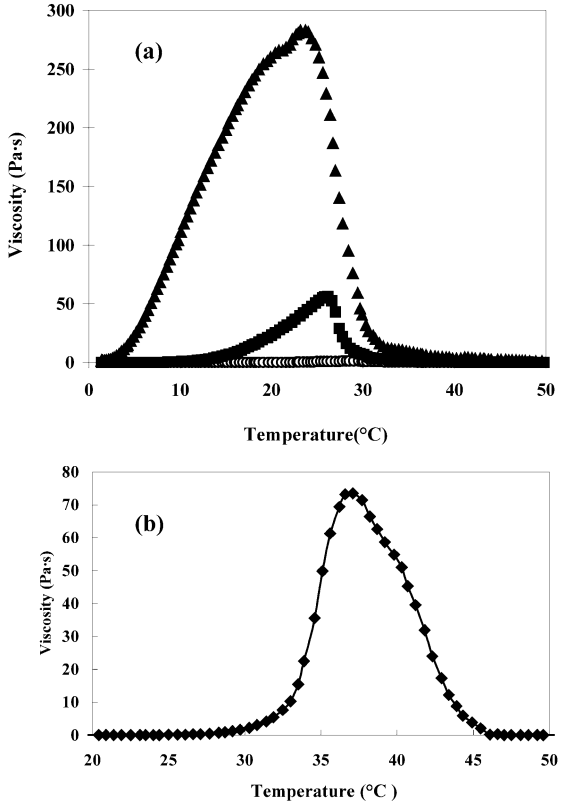


Fig. 2. (a) Viscosity change of 5 (○), 10 (■), and 20 wt% (▲) aqueous solutions of polymer **1** as a function of temperature under shear rate 1.7 s^{-1}. (b) Viscosity change of 10 wt% aqueous solution of polymer **2** (▲) as a function of temperature under shear rate 1.7 s^{-1}.

3.3. Thermosensitivity

It is well known that gelation of the thermosensitive polymers occurs via a physical cross-linking or a micellar aggregation mechanism, which is dependent on the polymer structure [23]. The gelation of the present polymers is also presumed to be attributed to the intermolecular hydrophobic interactions between the hydrophobic parts of the oligopeptide side groups, that is, ($-CH_2C_6H_5$) of PheEt, ($-CH(CH_3)-CH_2CH_3$) of IleEt, or ($-CH_2CH(CH_3)_2$) of LeuEt, which may form the strong physical junction in the polymer solution. Thus, the thermosensitivity of the present polymers is largely dependent on the structure of the oligopeptide, but the composition of the hydrophilic MPEG350 to the hydrophobic oligopeptide is very important.

It is surprising that polymer **1** and **2** bearing the equimolar side groups of the same hydrophilic MPEG350 and very similar tripeptides differing only in the terminal amino acids, leucine and isoleucine, show remarkable differences both in the gelation temperature and viscosity. The reason for such differences is not clearly understood, but it may be presumed that the isostructural leucine and

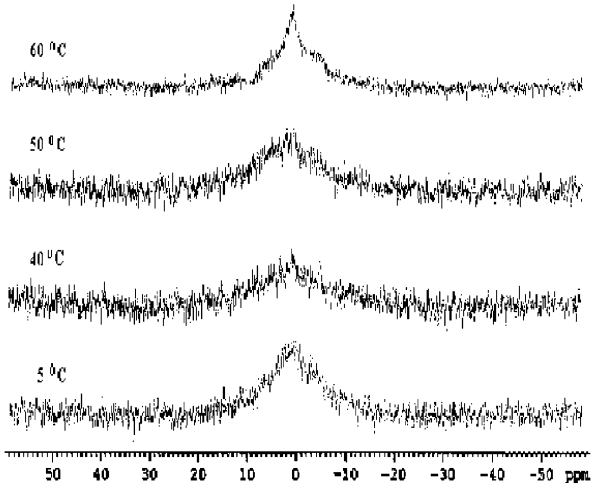


Fig. 3. Temperature-dependent ^{31}P NMR spectra of polymer **2** in D_2O (7.5 wt%).

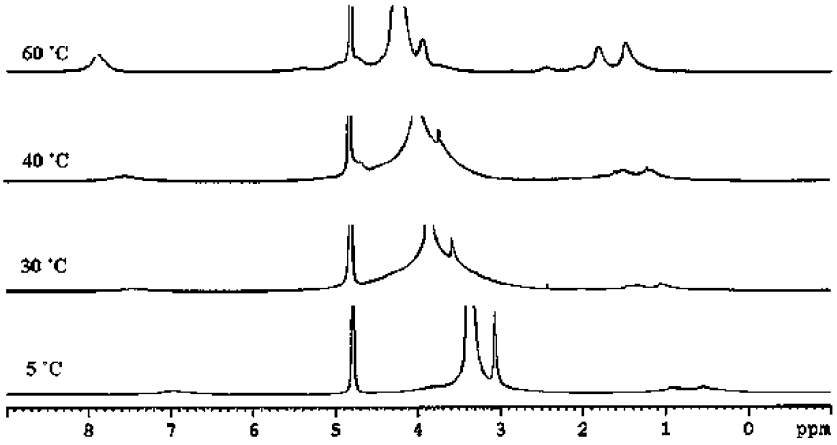


Fig. 4. Temperature-dependent ^{1}H NMR spectra of polymer **1** in D_2O (7.5 wt%).

isoleucine molecules when sequenced in the peptide can make a remarkable difference in the intermolecular hydrophobic interaction of their peptides probably due to the geometrical or conformational difference of their peptides, although they exhibit the same hydrophobicity. A similar example for the large difference in LCST due to the subtle difference in the structure of the hydrophobic alkyl groups was shown in the isostructural polymers of poly(*N*-isopropylacrylamide) [24].

The composition of the hydrophilic and hydrophobic side groups also affects greatly the thermal properties of the polymers, as seen in Table 1. The T_{ass}, T_{max}, and T_{lcst} values of the polymer solutions decrease with increasing content of the tripeptide: the T_{ass}, T_{max}, and T_{lcst} values of 32, 37, and 44 °C, respectively, for polymer **2** bearing 1.00 mol of GlyPheIleEt decreased to lower values of 12, 28, and 39 °C, respectively, for polymer **3** with 1.2 mol of GlyPheIleEt. It can be inferred from such results that increased hydrophobicity of the polymer decreases T_{ass}, T_{max}, and T_{lcst} values of the polymer, probably due to the increased intermolecular hydrophobic interactions, and as such the magnitude of the thickening process can be evaluated by the V_{max} values of the polymer solutions. For polymers **2** and **3** with the same side chains but different mole ratios, the higher content of GlyPheIleEt gave rise to the higher V_{max} value: the V_{max} values for polymers **2** and **3** were 73.5 and 330 Pa s, respectively. In contrast to polymers **2** and **3**, polymer **4** bearing high hydrophilic (1.1 mol MPEG350) to hydrophobic (0.9 mol GlyPheIleEt) balance did not form a gel and only showed a low critical solution temperature (LCST) at 50 °C. This result is probably attributed to lowered intermolecular hydrophobic interactions by the side group of GlyPheIleEt, which cannot form physical junctions strong enough to form a gel. Polymer **5** and **6** showed the same trend.

4. Conclusions

Biocompatible thermosensitive poly(organophosphazene) gels bearing MPEG and tri or tetrapeptide as side groups have been synthesized, and their sol–gel properties were investigated. The poly(organophosphazenes) in aqueous solution exhibited four-phase transitions with increasing temperature: a transparent sol, a transparent gel, an opaque gel, and a turbid sol. The gelation of the present polymers is presumed to be attributed to the intermolecular interaction between the hydrophobic oligopeptide side groups, which form strong physical junction zones in the polymer solution. The gelation properties of the present polymers were affected by the nature of the hydrophobic oligopeptide, composition of the hydrophilic and hydrophobic side groups, and the concentration of the polymer solutions. These polymers exhibit excellent thermogel properties useful for applications to injectable drug delivery and tissue engineering [25–28].

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