Synthesis of Poly[*N*-isopropylacrylamide-*g*-poly(ethylene glycol)] with a Reactive Group at the Poly(ethylene glycol) End and Its Thermosensitive Self-Assembling Character

MICHIHIRO IIJIMA,[1] YUKIO NAGASAKI[2]

[1]Department of Materials Chemistry and Bioengineering, Oyama National College of Technology, 771 Nakakuki, Oyama, Tochigi, 323-0806 Japan

[2]Tsukuba Research Center for Interdisciplinary Materials Science, University of Tsukuba, Tennoudai 1-1-1, Tsukuba 305-8453, Japan

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ABSTRACT: Poly[*N*-isopropylacrylamide-*g*-poly(ethylene glycol)]s with a reactive group at the poly(ethylene glycol) (PEG) end were synthesized by the radical copolymerization of *N*-isopropylacrylamide with a PEG macromonomer having an acetal group at one end and a methacryloyl group at the other chain end. The temperature dependence of the aqueous solutions of the obtained graft copolymers was estimated by light scattering measurements. The intensity of the light scattering from aqueous polymer solutions increased with increasing temperature. In particular, at temperatures above 40 °C, the intensity abruptly increased, indicating a phase separation of the graft copolymer due to the lower critical solution temperature (LCST) of the poly(*N*-isopropylacrylamide) segment. No turbidity was observed even above the LCST, and this suggested a nanoscale self-assembling structure of the graft copolymer. The dynamic light scattering measurements confirmed that the size of the aggregate was in the range of several tens of nanometers. The acetal group at the end of the PEG graft chain was easily converted to the aldehyde group by an acid treatment, which was analyzed by ^{1}H NMR. Such a temperature-induced nanosphere possessing reactive PEG tethered chains on the surface is promising for new nanobased biomedical materials.
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INTRODUCTION

In recent years, amphiphilic block and graft copolymers containing both a hydrophilic segment and a hydrophobic segment have been synthesized and extensively studied for their ability to form stable self-assemblies in selective solvents.[1] Nanoparticles formed from these self-assemblies are expected because of not only physicochemical interest but also nanobased materials science, especially biorelated science and engineering. Kataoka proved that a core–shell-type polymeric micelle of the size of several tens of nanometers, which had a hydrophilic poly(ethylene glycol) (PEG) as the outer shell and a hydrophobic core with an anticancer drug, reduced the toxicity of the drug and showed stable circulation in the blood stream

Correspondence to: Y. Nagasaki (E-mail: nagasaki@nagalabo.jp)
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for a prolonged time, eventually accumulating into a solid tumor[2–5] through the enhanced permeability and retention effect.[6,7] The important point of polymeric micelles as anticancer drug carriers is their virus-mimicking size (several tens of nanometers), which avoids not only renal excretion but also recognition by the reticuloendothelial system.[8] The PEG brushlike structure on the periphery of the sphere is one of the other important factors and prevents both the aggregation of particles with one another and the adsorption of biomolecules. Such stealth characteristics retard the opsonization of the nanosphere to improve circulation in the blood stream. A block copolymer micelle with an anticancer drug is now undergoing clinical tests in Japan. Recently, we have been focusing on the preparation of nanoparticles possessing reactive groups on the particle surface to provide the active targeting characteristic. For this design, we have synthesized several types of heterotelechelic poly(ethylene glycol)s (hetero-PEGs), which possess a functional group at one end and another functional group at the other chain end. With hetero-PEG as one of the segments of the amphiphilic block copolymer, a polymer micelle with a reactive group at the distal end of the PEG chain can be prepared.[9–11] Actually, our group reported that a sugar-installed core–shell-type polymeric micelle specifically interacted with the lectin.[12] These results indicate that a polymer micelle with a reactive group is promising as a high-performance active targeting drug vehicle.

Present demands for high-performance drug vehicles include controlling not only the targetability to an organ and cell but also traffic control in the cell. To improve the performance of the nanosphere vehicle, it is desirable to have several intelligent switches in the vehicle. There are several reports on the preparation of a nanosphere that changes its size and shape by a certain stimulus, such as pH[13] or temperature.[14–20] For example, Bae and coworkers[13] reported the preparation and evaluation of a sulfonamide-based, pH-sensitive polymeric micelle. Recently, we reported a pH-sensitive nanogel possessing reactive PEG tethered chains on the surface, which showed a volume transition around a neutral pH.[21] Okano's group[14] and Feijen's group[20] independently reported thermosensitive polymer micelles. More recently, a quite sophisticated nanomaterial system was reported by our group in terms of a DNA delivery system.[22] We synthesized a PEG/oligo-DNA block copolymer bearing an acidic–labile ester linkage between PEG and ODN. The sugar-installed PEG/ODN block copolymer coupled with a polycation formed a stable polyion complex. In addition to the tolerability against enzymatic degradation and minimal interaction with negatively charged biomacromolecules, the PEG–ODN/polycation polyion complex (PIC) micelles underwent a cleavage of the acidic–labile linkage between PEG and antisense ODN segments in response to endosomal pH, which is known to be 1.4–2.4 units lower than that under physiological conditions.[23] Thus, the design for an intelligent self-assembling system based on functional block copolymers is significantly progressing.

Graft copolymerization is one of the other ways of preparing desirable multicomponent polymeric systems. With a macromonomer technique,[24] it is easy to synthesize versatile types of graft copolymers. In contrast to the block copolymer self-assembling systems mentioned previously, however, there are few reports on intelligent self-assembling systems based on graft copolymers.[25]

We have so far been synthesizing hetero-PEG, which denotes PEG having a functional group at one end in addition to the polymerizable end group at the other chain end.[26] Hetero-PEG macromonomers are useful tools for creating an intelligent graft-copolymer system because the functional group can be installed at the end of the comb chain in the graft copolymer after copolymerization with specific comonomers. New intelligent designs can be feasible for this copolymerization system because versatile types of comonomers can be chosen. To expand this intelligent graft copolymer system, we designed a new graft copolymer having a thermosensitive segment as the main chain. In this article, the synthesis of poly[*N*-isopropylacrylamide-*g*-poly(ethylene glycol)] [poly(NIPAM-*g*-PEG)] possessing an aldehyde group at the PEG chain end and its thermal self-assembling characteristics are described (Fig. 1). The resulting nanosized self-assembling system is promising as a tool for intelligent drug carriers as well as nanodiagnostic systems.

EXPERIMENTAL

Materials

Commercial tetrahydrofuran (THF; Wako), benzene (Wako), 3,3-diethoxypropanol (Aldrich), ethylene oxide (EO; Sumitomo Seika), and methacrylic anhydride (Aldrich) were conventionally purified.[27] Potassium naphthalene was used as a THF solu-

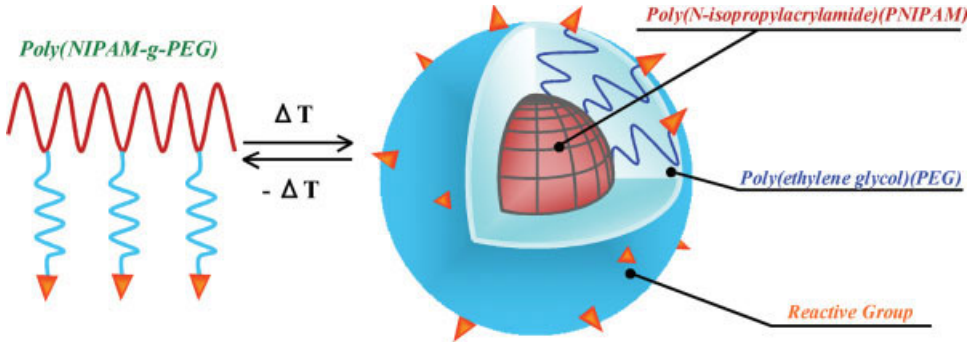


Figure 1. Schematic illustration of temperature-responsive poly(NIPAM-*g*-PEG) with a reactive group at the PEG end.

tion, the concentration of which was determined by titration. Benzoyl peroxide (BPO; Wako) was purified by recrystallization from a chloroform/methanol mixture. *N*-Isopropylacrylamide (NIPAM), which was kindly provided by Kohjin Co. (Japan), was recrystallized twice from *n*-hexane. All other chemicals were used as received.

Analysis

Size exclusion chromatography (SEC) measurements in organic solvents were carried out with a Toso HLC-8120 SEC instrument equipped with TSK SEC columns (TSKgel SuperHz 4000, SuperHz 3000, and SuperHz 2500). THF containing 0.5 wt % triethylamine was used as the eluent at a flow rate of 0.35 mL min^{-1} and 40 °C. SEC measurements for the fluorescent labeling experiments were carried out with a Toso HLC-8120 SEC instrument equipped with TSKgel columns (TSKgel SuperH 3000 and TSK guard column SuperH-L) and a fluorescent detector. Dimethylformamide (DMF) containing 10 mM lithium bromide was used as the eluent at a flow rate of 0.6 mL min^{-1} at 40 °C. The ^{1}H NMR spectra were obtained with a chloroform-*d* solution (1.0 wt %) with a JEOL EX400 spectrometer at 400 MHz. Chemical shifts with respect to $CHCl_3$ (1H:δ = 7.26) were employed. The gas chromatography measurements were carried out with an HP 5890 series II gas chromatograph equipped with a DB-1 (J&W Scientific) capillary column to calculate the conversion of NIPAM. A light-scattering spectrometer (DLS-7000 Photal, Otsuka Electronics) equipped with a 75-mW Ar laser that produces vertically polarized incident beams at $\lambda_0 = 488$ nm was used in this study for the dynamic and static light scattering measurements.

Polymer Synthesis

Synthesis of the α-Acetal-ω-methacryloyl-PEG Macromonomer

The α-acetal-ω-methacryloyl-PEG macromonomers were synthesized by the one-pot anionic ring-opening polymerization of EO with potassium 3,3-diethoxypropanolate (PDP) as the initiator at room temperature under argon. One of the representative procedures for the preparation of the α-acetal-ω-methacryloyl-PEG macromonomer is described. 3,3-Diethoxypropanol (10 mmol, 1.57 mL) and 10 mmol of potassium naphthalene were added to 60 mL of dry THF to form PDP. After a few minutes of stirring, 400 mmol (22.8 mL) of condensed EO was added via a cooled syringe to the formed PDP solution. The polymerization of EO proceeded for 2 days at room temperature and resulted in a highly viscous solution. After the polymerization, 50 mmol (3.10 mL) of a methacrylic anhydride was introduced into the polymer solution, and the reaction proceeded for a further 24 h. The polymer was recovered by precipitation into a 20-fold excess of cold isopropyl alcohol (−15 °C), stored for 2 h in a freezer, and centrifuged for 30 min at 5000 rpm. The polymer was then freeze-dried with benzene.

Synthesis of Poly(NIPAM-g-PEG) with an Acetal Group at the PEG Distal Chain End

Poly(NIPAM-*g*-PEG) possessing an acetal group at the PEG distal chain end was synthesized by the radical copolymerization of NIPAM with a hetero-PEG macromonomer with a redox initiator (BPO coupled with *N*,*N*-dimethylaniline) at room temperature under argon. One of the representative procedures for the preparation of poly (NIPAM-*g*-PEG) with the reactive PEG chains is described.

A 19.2-mmol (2.170 g) sample of NIPAM, 0.8 mmol (1.560 g) of the α-acetal-ω-methacryloyl-PEG macromonomer [number-average molecular weight (M_n) = 1950], and 0.8 mmol (0.194 g) of BPO were added to a 100-mL flask equipped with a three-way stopcock. The flask was degassed and purged with Ar. This cycle was repeated three times, and then 28.6 mL of dry benzene was added to the mixture in the flask to dissolve all the solids. The copolymerization was started by the addition of 1.6 mmol (0.20 mL) of *N,N*-dimethylaniline to the mixture solution and stirring for 44 h at room temperature, which resulted in a highly viscous solution. The mixture solution was then poured into a 20-fold volume of diethyl ether to precipitate the product. The collected polymer sample was subjected to freeze drying with benzene to remove the solvents employed in the synthesis.

Conversion of an Acetal Group at the PEG Chain End into an Aldehyde Group at the End of the PEG Chain in Poly(NIPAM-*g*-PEG)

The acetal group at the PEG chain end in poly (NIPAM-*g*-PEG) was converted to the aldehyde group by an acid treatment. Briefly, the aqueous solution (1 mg/mL) of the prepared graft copolymer was adjusted to pH 2 with HCl. After stirring for 1 h at room temperature, the polymer solution was transferred to a preswollen membrane tube (Spectra/Por molecular weight cutoff size = 12,000–14,000) and dialyzed against 2 L of water for 48 h to remove the salt. The dialysate water was exchanged at 4, 8, and 24 h from the beginning. A part of the polymer solution was frozen in liquid nitrogen and lyophilized for several measurements. The recovery of poly(NIPAM-*g*-PEG-aldehyde) [poly(NIPAM-*g*-PEG) possessing aldehyde group at the end of PEG chain] was about 90%.

Temperature Sensitivity of Poly(NIPAM-*g*-PEG) Aqueous Solutions

The temperature sensitivities of the graft copolymers in aqueous solutions were characterized by dynamic light scattering (DLS) measurements. The DLS measurements were performed with an argon-ion laser operating at a wavelength of 488 nm, an angular range of 30–150°, and temperatures from 20 to 60 °C. The concentration of the graft copolymer aqueous solution was adjusted to 1.0 mg/mL. Before each measurement, the sample cell was kept for 10 min at the test temperature. After the evaluation of the temperature sensitivity during the heating process, the samples were left overnight at room temperature to confirm the reproducibility. The polydispersity factor μ/Γ^2 was used to estimate the distribution of the obtained aggregates, where μ is the second moment of the distribution of the relaxation rates and Γ is the intensity-weighted mean relaxation rate. The ratio μ/Γ^2 is a measure of the width of the intensity distribution of the relaxation rates, which can be used to indicate the polydispersity of the sample under investigation.

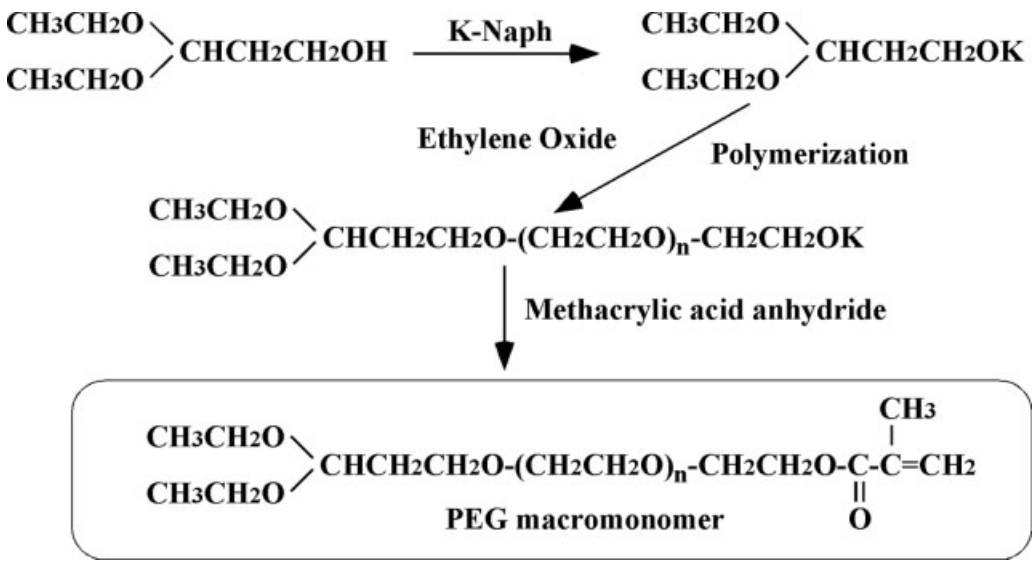
Fluorescent Labeling of the Aldehyde Group at the End of the PEG Chain of the Graft Copolymer

To confirm the conversion of the acetal group to the aldehyde group at the PEG chain end of the graft copolymer by the acid treatment, fluorescent labeling was carried out. To a 3 mg/mL acid-treated graft copolymer solution (60 mL), 50 mL of an 8-aminonaphthalene-1,3,6-trisulfonic acid disodium salt (ANTS) solution (2.4 mg/mL, 5.4 mmol/L) in phosphate buffered saline (PBS) (pH 8.0; *I*, ionic strength = 50 mM) was mixed and stirred for 30 min at the ambient temperature. After 0.81 mmol (0.051 g) of sodium cyanoborohydrate was added to the mixture, the mixture was further stirred for 90 min. The reacted polymer was recovered by dialysis with a preswollen membrane tube (Spectra/Pro; molecular weight cutoff size = 6000–8000) against 2 L of water for 4 days to remove any unreacted ANTS. The dialysate water was exchanged after 1, 2, and 3 days. The polymer was recovered by lyophilization after the polymer solution was frozen by liquid nitrogen. A fluorescent measurement was carried out with an SEC instrument equipped with a fluorescent detector at the excitation wavelength of 356 nm and the emission wavelength of 512 nm.

RESULTS AND DISCUSSION

Synthesis of the α-Acetal-ω-methacryloyl-PEG Macromonomer

For biomedical applications, PEG is widely used as a hydrophilic segment because of its biological suitability, including nonantigenicity and nontoxicity.[3,28,29] For the conjugation of PEG to substances (PEG-ylation reaction), semitelechelic PEG, which denotes PEG with a functional group at one end, is mainly used. To improve the utility of the PEG-ylated substrate, that is, to use the free



Scheme 1. Synthesis of a PEG macromonomer with a reactive group.

end of the conjugated PEG, heterobifunctional PEG (hetero-PEG), which denotes PEG with a functional group at one end and another functional group at the other end, is useful.

Recently, we have been focusing on facile and quantitative synthesis for the preparation of hetero-PEG.[30] Among the several types of hetero-PEGs, hetero-PEG with a polymerizable double bond at one end is useful for preparing graft copolymers with a functional group at the graft chain ends.[9,10,11,31,32] In this study, we designed acetal-PEG-methacrylate as a hetero-PEG macromonomer[33] because the acetal group can be easily converted to the aldehyde group by an acid treatment. The aldehyde group is useful for conjugation with biocomponents such as protein, amino sugar, and amino-ended-oligo-DNA via a reductive amination reaction. The synthesis of the α-acetal-ω-methacryloyl-PEG macromonomer was carried out with PDP with an acetal moiety as the initiator and methacrylic anhydride as the ω-end modification reagent (Scheme 1).

After the polymerization reaction, the reaction mixture was analyzed by SEC as shown in Figure 2(a). M_n and the weight-average molecular weight/number-average molecular weight ratio (M_w/M_n) of the polymerization product were 1950 and 1.05, respectively. M_n of the product agreed well with the one calculated with an initial monomer/initiator ratio {M_n = MW(EO)[[EO]$_0$/[PDP]$_0$] + MW(PDP) + MW(methacryloyl) = 44(40/1) + 148 + 69 = 1980}, indicating that PDP is the sole initiating species for this polymerization.

Figure 3 shows the ^{1}H NMR spectrum of the polymer after the purification. Signals based on both chain ends were clearly confirmed in the spectrum. The M_n value of PEG determined from ^{1}H NMR, assuming one acetal group per polymer, well agreed with the SEC results. The integration ratio of the acetal methine proton versus

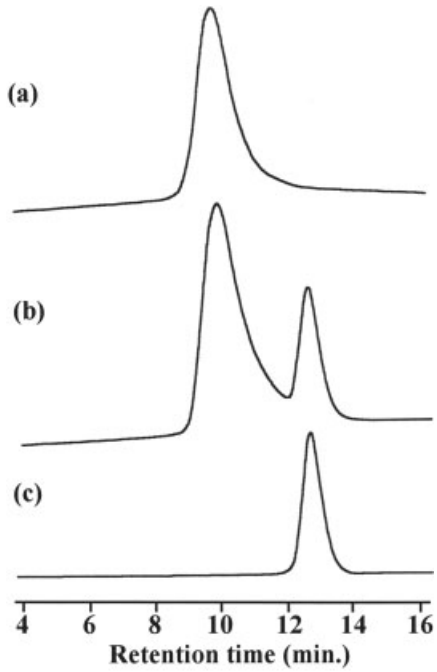


Figure 2. SEC of (a) poly(NIPAM-*g*-PEG) after purification, (b) poly(NIPAM-*g*-PEG) (sample 3), and (c) a PEG macromonomer with an acetal group.

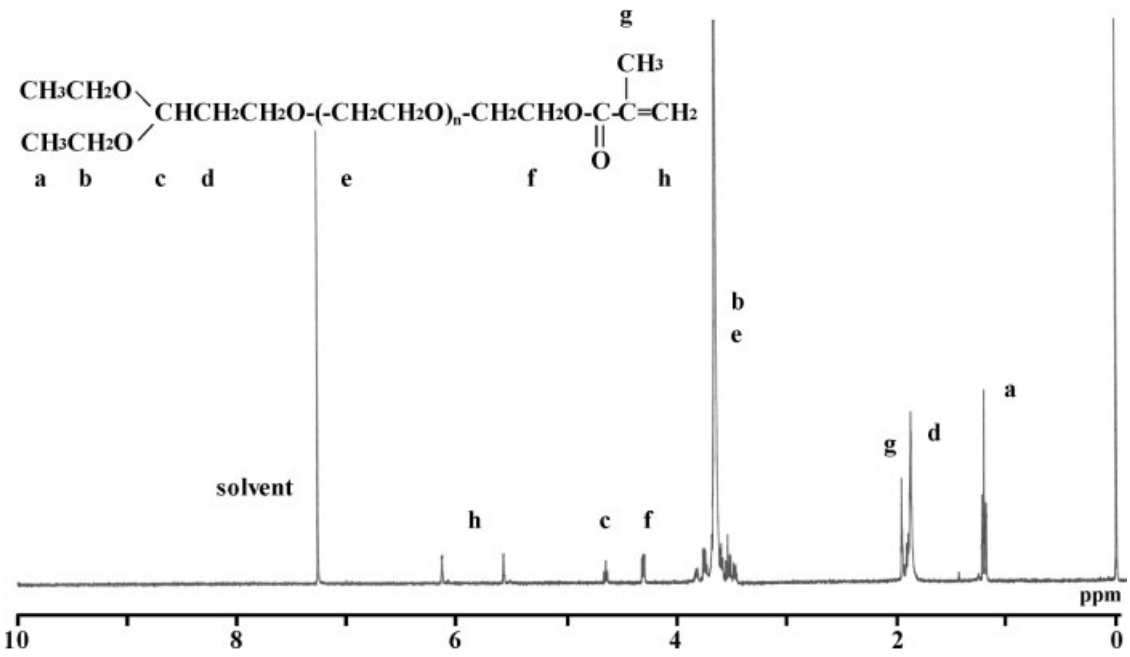


Figure 3. ^{1}H NMR spectrum of a PEG macromonomer with an acetal group in $CDCl_3$ (the same sample shown in Fig. 2(a)].

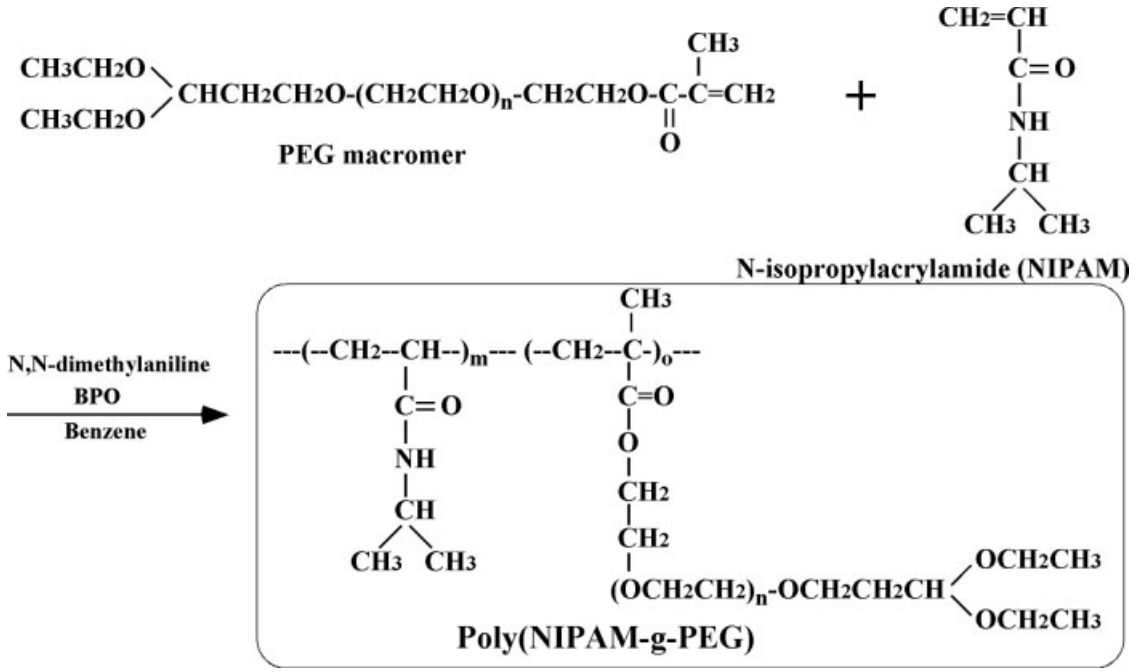
that of the vinyl β proton was almost unity, indicating the quantitative introduction of the ω-methacryloyl group. The yield of the polymer was 85%. On the basis of these results, the quantitative preparation of the hetero-PEG macromonomer was confirmed.

Synthesis of Poly(NIPAM-*g*-PEG) Possessing an Acetal Group at the PEG Distal Chain End

Recently, stimuli-sensitive polymers, which show a phase transition against a minute change in environmental stimuli such as the pH and temperature, have been paid very much attention in a variety of fields. For example, poly(*N*-isopropylacrylamide) (PNIPAM) shows a lower critical solution temperature (LCST) around 32 °C in aqueous media.[34] Because the phase-transition temperature of PNIPAM is close to body temperature, it has been widely investigated in biomedicine. Thus, many types of block and graft polymers, which have PEG as one of the segments, have been prepared.[17–20,35–38] To expand the utility of the PEG/PNIPAM copolymers, the copolymerization of NIPAM with hetero-PEG macromonomers was carried out. The resulting polymers should possess a reactive group at the distal end of the PEG graft chains and can be used as highly functional biomaterials such as drug delivery systems, cell cultures, isolators of biomolecules, membranes, and enzyme activity controllers.

With hetero-PEG macromonomers possessing an acetal group at one end, the poly(NIPAM-*g*-PEG) graft copolymers were prepared. The acetal group is known to react with radicals; that is, the radical abstracts the methine hydrogen to produce a radical coupling reaction, which may cause serious side reactions, such as a crosslinking reaction. To avoid such an undesired side reaction, we employed a redox copolymerization system at a rather low polymerization temperature, as shown in Scheme 2.

After the redox polymerization, the mixture became a highly viscous liquid but retained its transparency. This indicates that no gel was formed, and a water-soluble polymer was produced in the reaction mixture. From the SEC measurement of the reaction mixture, as shown in Figure 2(b), it was confirmed that copolymerization took place, although the PEG macromonomer remained to some extent. After precipitation in diethyl ether and freeze drying with benzene, the unreacted PEG macromonomer was completely removed, as shown in Figure 2(c). No shoulder was observed in the higher molecular weight region of the peak, and this indicated that no remarkable reaction occurred between the preformed polymers. From the ^{1}H NMR spectrum of the polymer after purification, as shown in Figure 4(a), typical signals based on both PNIPAM (e.g., 4.0 ppm) and PEG



Scheme 2. Synthesis of poly(NIPAM-*g*-PEG) with an acetal group at the PEG end.

(e.g., 3.6 ppm) segments appeared. On the basis of these two results, that is, SEC and NMR, poly (NIPAM-*g*-PEG) was properly synthesized. The signals assignable to the acetal proton at 4.6 ppm remained intact, and this indicated that no remarkable side reaction took place around the acetal group.

The results of the redox copolymerization reaction of NIPAM with hetero-PEG macromonomers under several reaction conditions are summarized in Table 1. The PEG content of the graft copolymers was determined from the ^{1}H NMR spectra. The average number of grafted PEGs on each PNIPAM chain backbone was determined from the PEG content and M_n.

Conversion of the Acetal End Group at the PEG Chain End of Poly(NIPAM-*g*-PEG) into an Aldehyde Group

The conversion of the end acetal group to the aldehyde group in poly(NIPAM-*g*-PEG) was carried out by the addition of HCl to an aqueous polymer solution (1.0 mg/mL). After a specific period of time, the reaction was quenched by neutralization with aqueous NaOH, and the graft copolymer was purified by dialysis. The conversion reaction of acetal into aldehyde was monitored by ^{1}H NMR of the polymer after freeze drying from water. As shown in Figure 4(b), the acetal methine proton at 4.6 ppm completely disappeared, whereas a new peak at 9.8 ppm, which was assigned to the aldehyde proton, clearly appeared. From the ^{1}H NMR spectrum, more than 90% of the acetal group was converted into aldehyde by the reaction.

Temperature Sensitivity of Poly(NIPAM-*g*-PEG) Aqueous Solutions

Because PNIPAM possesses an LCST around 32 °C, the obtained graft copolymers also anticipated the phase-transition characteristics. To evaluate the temperature sensitivities, a DLS analysis of aqueous solution of the obtained graft polymer was carried out at various temperatures. Figure 5 shows the temperature dependency of the scattering intensity of the poly(NIPAM-*g*-PEG) solutions. At temperatures lower than 40 °C, the graft copolymer solutions prepared in this study showed a very low scattering intensity, which indicated that the polymer was molecularly dispersed. On the contrary, the scattering intensity of the solutions (except sample 2) significantly increased above 40 °C, indicating the self-assembling of the graft copolymers. In the case of sample 2, the scattering intensity did not increase significantly even above the LCST of PNIPAM. Tenhu et al. reported that a copolymer of PNIPAM with PEG having a PEG concentration of more than 30 wt % caused a decreased phase-transition tendency. Sample 2 was thought to have a similar tendency.[17–19]

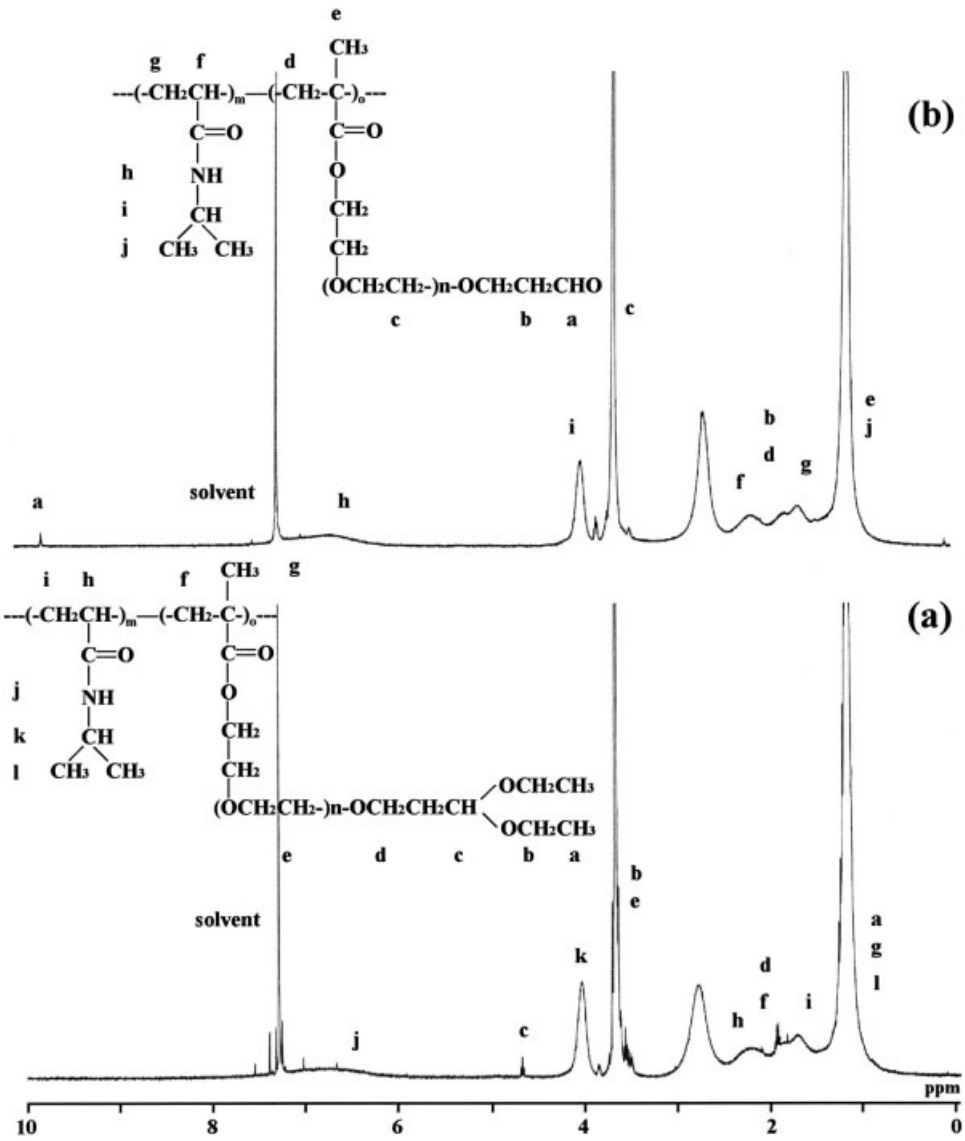


Figure 4. ^{1}H NMR spectrum of poly(NIPAM-*g*-PEG) with an acetal group at the PEG end in $CDCl_3$ (sample 3) (a) before and (b) after hydrolysis by an acid treatment.

Regardless of the scattering intensity variations, the graft copolymer solutions were completely transparent, and this suggests no large aggregates even in the self-assembling structures. Information on the size and shape were obtained from the DLS measurements. The dependence of the scaled characteristic line width ($\Gamma/\mathbf{K}^2$; the diffusion coefficient) on the scattering vector ($\mathbf{K}^2$), which corresponds to the scattering angle, is shown in Figure 6. It has been reported that the $\Gamma/\mathbf{K}^2$ values are independent of $\mathbf{K}^2$ in the case of spherical particles, whereas a particle with anisotropy shows strong angular dependency, which is based on the rotational motions.[39] As shown in the figure, the angular dependency of sample 3 was negligible, whereas sample 2 showed a strong angular dependency. The results indicated that the graft copolymer possessing a PEG concentration lower than 30 wt % (sample 3) showed the compact packing form at the temperature above the LCST because of the segregation of the hydrophobic PNIPAM segments. On the contrary, the lower segregation force of sample 2 might cause a loose association of the polymers even above the LCST of the PNIPAM segments. The improvement in the hydrophilicity of the graft copolymer as well as the steric interactions of the PEG chains might be the most significant factors. Under the suitable balance of PEG graft chains against the PNIPAM main chain, an ideal nano-

Table 1. Results of the Radical Copolymerization of NIPAM with a PEG Macromonomer Having an Acetal Group at the End[a]

Sample	Monomer		Monomer Concentration (mol/L)	$[M]_0/[I]_0$[b]	Reaction Time (h)	Conversion of NIPAM (%)[c]	Yield (%)	PEG/NIPAM (Molar Ratio) in the Polymer[d]	PEG Content (wt %)[e]	$10^{-3}\ M_n$	M_w/M_n	N_{PEG}[f]
	PEG	NIPAM										
1	0.4	11.6	0.8	100	64	94.6	25	1:50	25.7	17.3	2.12	2.3
2	1.6	18.4	0.8	100	24	100	68.6	1:18	48.9	22.9	5.36	5.7
3	0.8	19.2	0.7	25	44	100	65.2	1:38	31.2	14.6	2.14	2.3
4	0.8	19.2	0.7	12.5	44	100	67.0	1:45	27.7	12.3	2.04	1.8

[a] Solvent = benzene; temperature = room temperature; M_n of PEG = 1950.
[b] $[M]_0$ = initial monomer concentration, $[I]_0$ = initial initiator concentration.
[c] Determined from gas chromatography results.
[d] Determined from ^{1}H NMR results.
[e] Determined from gel permeation chromatography results.
[f] The average number of grafted PEGs on each PNIPAM chain backbone determined from both ^{1}H NMR and GPC data.

Figure 5. Light scattering intensity of poly(NIPAM-*g*-PEG) aqueous solutions (1 mg/mL) as a function of temperature: (■) sample 1, (▲) sample 2, (◇) sample 3, and (□) sample 4.

scale self-assembling structure could be formed even by the graft copolymers.

Figure 7 shows typical examples of the size distribution based on the histogram analysis. The weight-average diameter and *dw*/*dn* values of sample 3 at 60 °C were 26.0 nm and 1.20, respectively. A very small size with a narrow size distribution was confirmed in sample 3. Figure 8 shows the change in the size of the self-assembled particles as a function of the temperature above the LCST of PNIPAM. It was confirmed that the size of all the samples was in the range of 20–90 nm, regardless of the temperature. With increasing temperature, the tendency was observed

Figure 6. Plots of the $\mathbf{K}^2$-scaled average characteristic line width Γ, ($\Gamma/\mathbf{K}^2$) versus $\mathbf{K}^2$ for poly(NIPAM-*g*-PEG) aqueous solutions at 60 °C: (▲) sample 2 and (◇) sample 3.

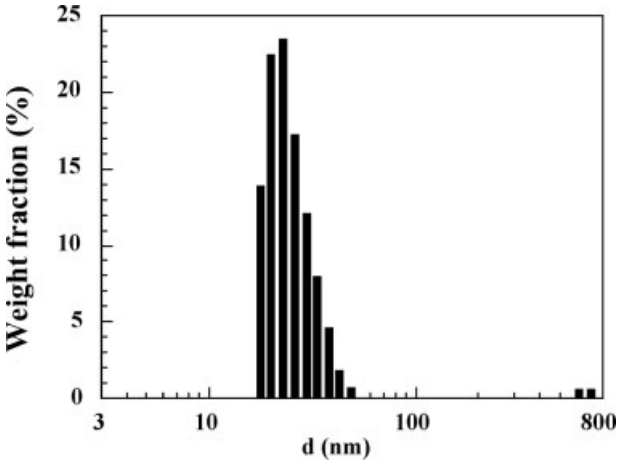


Figure 7. Size distribution of poly(NIPAM-*g*-PEG) determined by a DLS histogram analysis (sample 3). d = diameter.

that the samples having the lower PEG concentration were reduced in size. PEG is known to possess a cloud point at a high temperature.[40] In this region, the shrinking of the PEG chain on the collapsed PNIPAM core might take place above the LCST of PNIPAM. Wu et al.[41] reported that the size of a self-aggregate decreased with increasing temperature from 40 to 50 °C in a PNIPAM-*g*-PEG possessing a high PEG content. In our case, however, a remarkable change in the size was not observed in the case of the graft copolymer with a high PEG concentration (sample 3). The end carboxylate group may be responsible for the difference in these results.

A ^{1}H NMR analysis of the sample 3 solution was carried out as a function of temperature. As shown in Figure 9, the proton signals based on the PNIPAM segment—the methine proton at 4.0 ppm, the methylene protons around 1.5–2.5 ppm, and the methyl protons at 1.2 ppm—gradually decreased with increasing temperature, and this indicated that the PNIPAM segments collapsed and caused a broadening of these signals due to the restricted mobility in the NMR spectroscopy. On the basis of these results, that is, the increase in the scattering intensity, the decrease in its size, and the decrease in the proton signals based on the PNIPAM main chains with increasing temperature, the nanosized spherical self-assembling structure of poly(NIPAM-*g*-PEG) was confirmed with the suitable compositions.

To confirm the usefulness of the end group of the graft copolymers, the introduction of a fluorescent probe to the PEG chain end was carried out. After the acetal group at the end of the PEG chain end was converted to the aldehyde group by the acid treatment, ANTS was added under reducing conditions. ANTS is known to selectively react with the aldehyde group via the reductive amination reaction. The installation of the fluorescent probe was confirmed with the SEC instrument equipped with a fluorescent detector. Figure 10 shows the SEC profiles before and after the fluorescent labeling. No fluorescent peak was observed before the ANTS installation. After the installation reaction, on the contrary, a strong fluorescent peak was observed, indicating the effective installation of the fluorescent probe at the PEG chain end via the reductive amination reaction. Because many types of biocomponents such as antibodies, enzymes, inhibitors, and oligonucleotides possess a primary amino group, poly(NIPAM-*g*-PEG), possessing the aldehyde group at the graft chain end, can be used as an intelligent bioconjugation agent.

CONCLUSIONS

The radical copolymerization of NIPAM with hetero-PEG macromonomers, which possess an acetal group at one end and a methacryloyl group at the other chain end, was carried out with a redox initiation system. The copolymerization homogeneously proceeded without gel formation. The obtained graft copolymers having a PEG concentration lower than 30% formed self-assembled

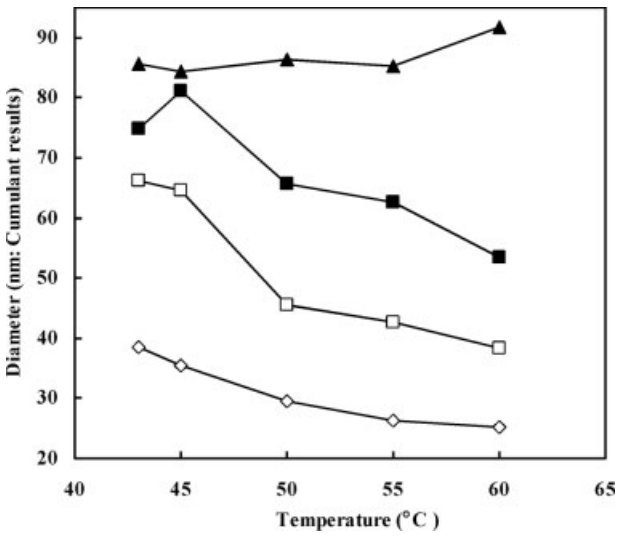


Figure 8. Change in the cumulant diameter of poly (NIPAM-*g*-PEG) aqueous solutions (1 mg/mL) as a function of temperature: (■) sample 1, (▲) sample 2, (◇) sample 3, and (□) sample 4.

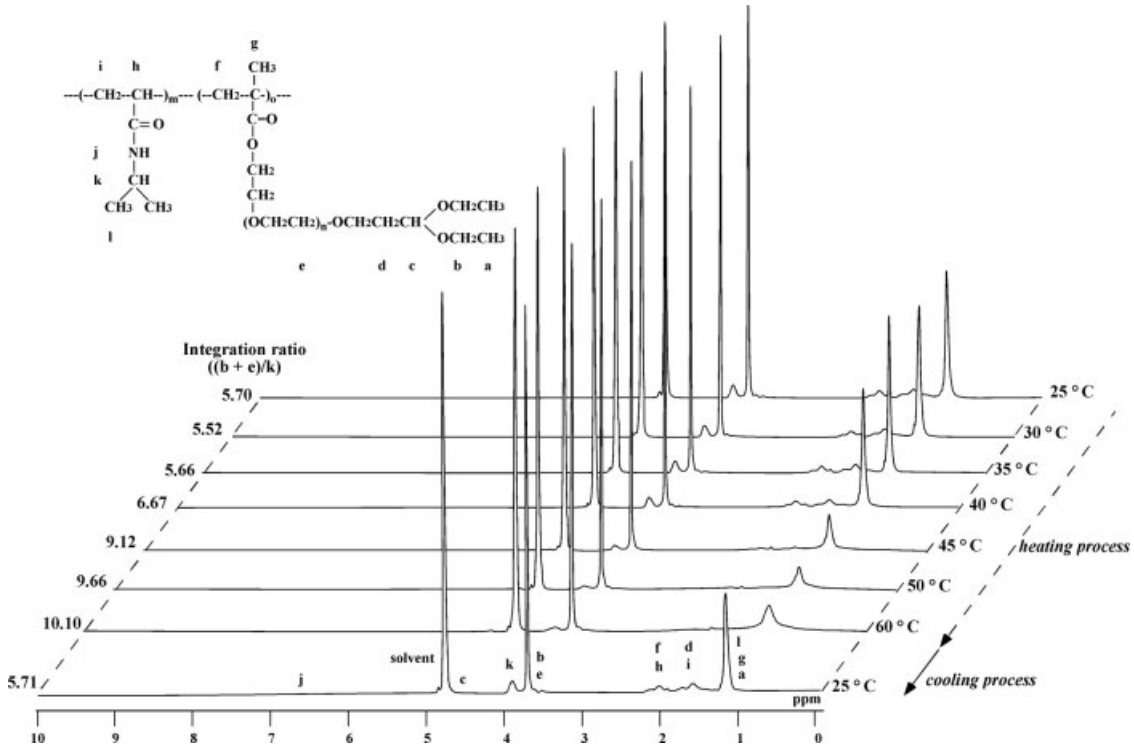


Figure 9. ^{1}H NMR spectra of a poly(NIPAM-*g*-PEG) solution (sample 3) in D_2O at a variety of temperatures [the methylene proton at 3.8 ppm (PEG main chain) was used as a standard peak, and the relative intensities were plotted].

structures of the ideal nanoscale type along with a narrow size distribution at temperature above 40 °C, as confirmed by DLS analysis. ^{1}H NMR measurements confirmed that the PNIPAM main chain became a solid core, retaining the nanosized shapes. The fluorescent probe was confirmed to be introduced at the end of the PEG graft chain via reductive amination reactions. Such a temperature-induced nanosphere possessing reactive PEG tethered chains on its periphery is promising as a new nanobased biomedical material.

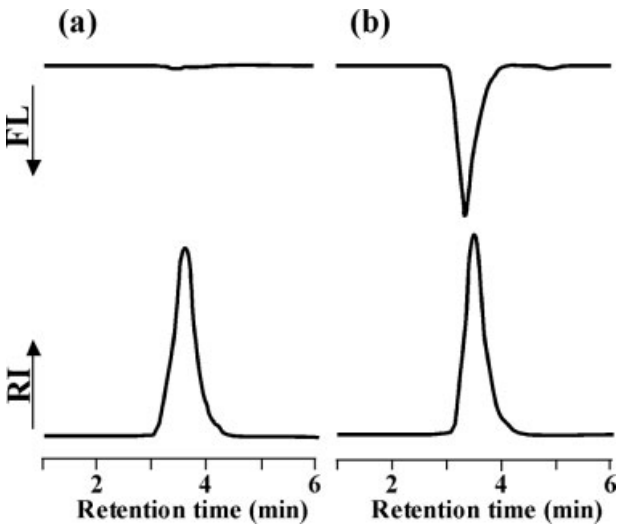


Figure 10. SEC profiles of poly(NIPAM-*g*-PEG)s (sample 3) in DMF (a) before and (b) after the fluorescent (FL) labeling (excitation wavelength = 356 nm, emission wavelength = 512 nm). RI: refractive index.

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