Preparation of pendant group-functionalized amphiphilic diblock copolymers in the presence of a monomer activator and evaluation as temperature-responsive hydrogels

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**Abstract**

Temperature-responsive hydrogels have been widely developed for biomedical applications. In this study, 4-benzyloxy-\(\varepsilon\)-caprolactone (CL-OBn) was synthesized and the subsequent ring opening polymerization (ROP) of \(\varepsilon\)-caprolactone (CL) and CL-OBn monomers was performed by using the hydroxyl group of methoxy polyethylene glycol (MPEG) in the presence of HCl Et2O as a monomer activator. MPEG-b-poly (\(\varepsilon\)-caprolactone) (MC) with benzyl pendant groups (MC-OBn) was successfully obtained in almost quantitative yield and the segment ratios and molecular weights were close to the theoretical values. Deprotection of the benzyl pendant groups successfully provided MC with hydroxyl pendant groups (MC-OH). Suspensions of MC-OBn and MC-OH exhibited distinct sol-to-gel phase transitions as a function of the temperature. The sol-to-gel phase transition of the MC-OBn and MC-OH diblock copolymers depended on the type (benzyl or hydroxyl) and amount of pendant groups in the diblock copolymer structure. The pendant group on the MC-OBn and MC-OH diblock copolymers either stabilized and/or destabilized hydrophobic aggregation between the MC segments. The hydrophobic aggregation was confirmed by the detection of crystallinity in the MC-OBn and MC-OH diblock copolymer suspensions. In conclusion, MC-OBn and MC-OH diblock copolymers were successfully prepared and served as temperature-responsive hydrogels.

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1. Introduction

Temperature-responsive hydrogels exhibit sol-to-gel phase transitions between room temperature and body temperature [1,2]. Such temperature-responsive hydrogels have attracted great attention in the past few decades for application in injectable biomedical systems. Several cells and/or bioactive molecules can easily be entrapped in a formulation that is an injectable solution at room temperature solution, where the solution can be easily injected into the body by using a syringe and becomes a hydrogel at the injected site [3–6].

Several kinds of amphiphilic block copolymers consisting of hydrophobic biodegradable aliphatic polyesters, poly (l-lactic acid) (PLLA), poly (glycolic acid), or poly (\(\varepsilon\)-caprolactone) (PCL) and their copolymers, and hydrophilic poly (ethylene glycol) (PEG) have been reported as typical examples of temperature-responsive injectable hydrogels [7–9]. The driving force for temperature-responsive hydrogels of amphiphilic block copolymers consisting of PEG and polyesters is the formation of a structured network via hydrophobic aggregation between the hydrophobic polyester blocks.

It is well known that the ring opening polymerization (ROP) of ester monomers [such as l-lactic acid (LA), glycolic acid (GA), and \(\varepsilon\)-caprolactone (CL)] leads to the formation of amphiphilic block copolymers in the presence of stannous octoate or organometallics as a monomer activator or catalyst at comparatively high temperatures [10,11]. Metal catalyst should be one of the concerns for the use of temperature-responsive hydrogels of amphiphilic block copolymers for biomedical application.

Thus, it is necessary to develop a metal-free ROP to copolymerize LA, GA or CL. Even though some efforts were devoted to polymerize LA, GA or CL by using organic catalysts or without...
catalyst for long polymerization time [12–15], there are a limited studies of the polymerization as the metal-free method.

Recently, Endo et al. reported successful ROP of CL with alcohol initiator in the presence of HCl Et2O as the metal-free method [16,17]. Some researchers have also studied the feasibility of the ROP of CL and LA ester monomers using the polymers with hydroxyl group as the initiator at room temperature in the presence of HCl Et2O [18–20] or trifluoromethanesulfonic acid [21–23] as a monomer activator to prepare amphiphilic block copolymers. In this polymerization process, the monomer activator could be easily removed from the amphiphilic block copolymers after ROP. Additionally, ROP could be performed at room temperature. Thus, ROP in the presence of HCl Et2O or trifluoromethanesulfonic acid is one of the most powerful as the metal-free method of preparing amphiphilic block copolymers.

More recently, we prepared amphiphilic diblock copolymers such as MPEG–b–PCL (MC), MPEG–b–polyester, MPEG–b–(PCL–ran–poly (trimethylene carbonate)), and MPEG–b–(PCL–ran–poly (1,4-dioxan-2-one)) through ROP in the presence of HCl Et2O and reported the formation of temperature-responsive hydrogels [24–26].

Generally, mechanism of the hydrogel formation of amphiphilic block copolymers can be proposed by the micelle percolation. The amphiphilic block copolymers in aqueous solution were firstly formed into micelles, then aggregation of micelles and finally hydrophobic aggregated domains.

If amphiphilic block copolymers have functional group at the chain end or pendant-position, the functional groups on amphiphilic block copolymers can alter the packing or aggregation in the micelle formation and then affect the occurrence of the macroscopic gelation through stabilization or destabilization of intra- and inter-molecular hydrophobic aggregation.

Several amphiphilic block copolymers with various functional groups in the chain end- or pendant-position have also been prepared [27–33]. The prepared amphiphilic block copolymers with various functional groups were utilized as the temperature-responsive hydrogel or micelles for preparation of nanoparticles, or drug and gene delivery carriers [34–37].

Recently, Hedrick et al. reported the amphiphilic block copolymers with various functional groups by ROP of several cyclic ester monomers containing benzyl, hydroxyl, amino and carboxyl groups in the presence of stannous octoate [38–40]. More recently, Lee et al. reported the preparation of MPEG–b–PCL (MC) with benzyl carboxylate, carboxylic or hydroxyl groups by ROP using stannous octoate at comparatively high temperature and then examined only micellar characteristics [41–44].

Meanwhile, we also prepared MC with a carboxylic acid group, an amine group, and a zwiterionic group at the chain end-position of the MC chain and examined the temperature-responsive properties [45,46]. Additionally, we reported temperature-responsive injectable hydrogels of MPEG–b–(PCL–ran–PLLA) (MCL) amphiphilic diblock copolymers modified with benzyl, hydroxyl, or carboxylic acid groups at the chain pendant-position of MCL [47]. The preparation of MCL functionalized at the chain pendant-position was achieved by ROP of the 3-benzyloxyxymethyl- 6-methyl-1,4-dioxan-2,5-dione (ILA) monomer with a benzyloxyethyl group at the 3-position of the lactide, with subsequent derivatization. However, the total yield of ILA was quite low, being below 20%. In addition, ROP of ILA proceeded at comparatively high temperature with stannous octoate. Although some studies have been reported the preparation of amphiphilic block copolymers with various functional groups in the chain end- or pendant-position, in this work our interest has focused on two aims. Firstly, we tried to prepare pendant group-functionalized amphiphilic diblock copolymers in the room temperature by the use of metal-free method. Secondly, we examined the behavior as temperature-responsive injectable hydrogels of the prepared pendant group-functionalized amphiphilic diblock copolymers.

Hence, we considered 4-benzyloxy-ε-caprolactone (CL-OBn) as a candidate for achieving facile ROP in the presence of HCl Et2O as a monomer activator. Accordingly, in this study, we address the following questions: 1) Can ROP of CL and OBn in the presence of HCl Et2O using the hydroxyl group of MPEG as an initiator at room temperature (as the metal-free method) provide MPEG–b–poly (ε-caprolactone–ran–poly (4-benzyloxy-ε-caprolactone) (MC–Cl–ran–CL–OBn)) (MC–OBn) amphiphilic diblock copolymers and then can MPEG–b–poly (ε-caprolactone–ran–poly (4-hydroxy-ε-caprolactone) (MC–Cl–ran–CL–OH)) (MC–OH) be prepared by subsequent deprotection reaction of benzyl group? 2) Can the prepared MC–OBn and MC–OH be used as temperature-responsive hydrogels? Elucidation of these issues will have a basic understanding on the effects by the type and content of pendant groups on amphiphilic block copolymers.

2. Experimental

2.1. Materials

Sodium borohydride (NaBH4), sodium hydride (NaH), benzyl bromide (BnBr), 4-chloroperbenzoic acid (mCPBA), 10 wt% polallodium on carbon (Pd/C), triethylamine (TEA), and 4-(dimethylaminopyridine) (DMAP) were used as received from Aldrich (MO, USA). Methanol and 1,4-dioxane were used as received from J.T. Baker (PA, USA). Tetrahydrofuran (THF), ethyl acetate (EA), magnesium sulfate (MgSO4), dichloromethane (CH2Cl2), n-hexane, ethyl ether and hydrochloric acid (HCl: 35.0–37.0% in DW) were used as received from Samchun (Gyeonggi, Korea). HCl (Aldrich; 1.0 M solution in diethyl ether, MO, USA), 1,4-cyclohexanediene monoethylene ketel (TCl, Tokyo, Japan) and MPEG (number-average molecular weight Mn = 750, Aldrich, Milwaukee, WI, USA) were used as received. ε-Caprolactone (CL; Aldrich, Milwaukee, WI, USA) was repeatedly distilled over calcium chloride (CaCl2; Aldrich, MO, USA) under nitrogen before use.

2.2. Characterization

For analysis of the MC, MC-OBn, and MC-OH diblock copolymers, 1H-NMR and 13C-NMR spectra were acquired on a Varian Mercury Plus 400 (Varian) instrument using (CD3)2CO or CDCl3 in the presence of TMS as an internal standard. A YL-Clarity GPC system (YL Instruments, Gyeonggi, Korea) equipped with YL1970 RI detector and three columns (Shodex K-802, K-803, and K-804 poly styrene gel columns) at 40 °C was used to determine the molecular-weight distributions of the diblock copolymers. In the measurement, the flow rate of the CHCl3 eluent was 1.0 mL/min. Differential scanning calorimetry (DSC; Q1000, TA Instruments, Germany) was used to determine the melting temperature (Tm) and the heat of fusion (ΔHm) of the MC, MC-OBn, and MC-OH diblock copolymer solutions. DSC analysis of the MC, MC-OBn, and MC-OH diblock copolymers in aqueous solutions was performed from 10 to 60 °C at a heating rate of 5 °C/min under nitrogen atmosphere. The heat of fusion per gram of diblock copolymer was determined from the area under the peak.

2.3. Preparation of 4-benzyloxy-ε-caprolactone (CL-OBn)

2.3.1. Preparation of 1,4-cyclohexanediene monoethylene ketel

All glasses were heated under vacuum and then flushed with a dry nitrogen stream. All reactions were conducted under a dry
nitrogen stream. 1,4-Cyclohexanedione monoethyleneketal (13 g, 83.24 mmol) was dissolved in methanol (200 mL) and stored at 0 °C. NaBH₄ (3.78 g, 99.89 mmol) was added to the solution, followed by stirring. After 12 h, the resulting solution was concentrated by rotary evaporation to remove the methanol. The reaction mixture was then washed with deionized water (DW) and ethyl acetate (EA) three times. The organic phase was dried over anhydrous MgSO₄ and concentrated by evaporation, yielding (3) (8.67 g, 42.44 mmol, 95%) as a yellowish viscous liquid. ¹H-NMR ((CD₃)₂CO): 2.05 (s, 1H, -CH₂(Cl)-), 3.91 (t, 2H, -OCH₂-), 3.91 (t, 2H, -OCH₂-), 1.80 (m, 4H, -CH₂-, -CH₂-), 1.59 (m, 4H, -CH₂-).

2.3.2. Preparation of 1-ethylene acetal-4-benzyloxy-cyclohexane
1,4-Dioxaspiro-4.5-decan-8-ol (1) (11.7 g, 73.96 mmol) was dissolved in anhydrous THF (65 mL) and stored at room temperature. Aqueous HCl (30 mL; 4 N) was added to the solution that was then stirred for 12 h. The reaction solution was concentrated by rotary evaporation to remove the THF. A solution was neutralized by adding sodium bicarbonate solution. The resulting solution was concentrated by rotary evaporation and dried under vacuum yielding (1) 11.7 g, 78%) as a yellowish liquid. ¹H-NMR ((CD₃)₂CO): 2.05 (s, 3H, -CH₂(Cl)-), 3.91 (t, 2H, -OCH₂-), 3.91 (t, 2H, -OCH₂-), 1.82 (m, 4H, -CH₂-, -CH₂-), 1.59 (m, 4H, -CH₂-).

2.3.3. Preparation of 4-benzyloxy-cyclohexanone
1-Ethylene acetal-4-benzyloxy-cyclohexane (2) (9.13 g, 36.75 mmol) was dissolved in anhydrous THF (65 mL) and stored at room temperature. Aqueous HCl (30 mL; 4 N) was added to the solution that was then stirred for 2 h. BnBr (9.66 mL, 56.50 mmol) in anhydrous THF (130 mL) was added to the solution at 0 °C at a rate of 5 mL/min, followed by stirring for 24 h. The reaction was quenched by addition of 5 mL DW. The resulting solution was concentrated by rotary evaporation to remove the THF. The reaction mixture was washed with DW and EA three times. The obtained mixture was purified by silica-gel column chromatography using a solution of n-hexane and EA (v/v = 40/10) as the eluent, yielding (2), (9.13 g, 36.75 mmol, 78%) as a yellowish liquid. ¹H-NMR ((CD₃)₂CO): 2.05 (s, 3H, -CH₂(Cl)-), 3.91 (t, 2H, -OCH₂-), 3.91 (t, 2H, -OCH₂-), 1.82 (m, 4H, -CH₂-, -CH₂-), 1.59 (m, 4H, -CH₂-).

2.3.4. Preparation of 4-benzyloxy-ε-caprolactone (CL-OBn)
MCPOA (14.66 g, 84.89 mmol) was dissolved in anhydrous CH₂Cl₂ (60 mL) and kept at room temperature. 4-Benzoyloxy-cyclohexanone (8.67 g, 42.44 mmol) was added to the solution, followed by stirring for 24 h. The reaction solution was filtered through celite. The resulting solution was concentrated by rotary evaporation to remove the CH₂Cl₂. The reaction mixture was washed with DW and sodium bicarbonate solution three times. The organic phase was dried over anhydrous MgSO₄ and concentrated by evaporation. The obtained mixture was purified by silica-gel column chromatography using a solution of n-hexane and EA (v/v = 40/10, Rf = 0.13) as the eluent, yielding (4, CL-OBn), (5.90 g, 26.77 mmol, 68%) as a clear liquid. ¹H-NMR ((CD₃)₂CO): 2.05 (s, 3H, -CH₂(Cl)-), 2.53 (t, 2H, -OCH₂-), 3.69 (m, 1H, -OCH-), 3.01, 2.41 (t, 2H, -OCH₂-), 2.00 (m, 4H, -CH₂-, -CH₂-). Anal. Calcd. for C₁₉H₁₆O₃, C: 70.89, H: 7.32. Found: C: 70.36, H: 7.32.

2.4. Synthesis of MPEG-b-PCL diblock copolymer (MC)
MC diblock copolymers of PCL (molecular weight: 2416 g/mol) and MPEG (750 g/mol) were prepared via a previously reported block copolymerization method [20].

2.5. Synthesis of MPEG-b-[poly(ε-caprolactone)-ran-poly(4-benzyloxy-ε-caprolactone)] (MC-OBn)
All glasses were heated under vacuum and dried via flushing with a dry nitrogen stream. A typical polymerization process was used to generate MC-(OBn)₅ with a CL/CL-OBn ratio of 95/5 using MPEG (750 g/mol) as an initiator, as follows: MPEG (3.13 g, 4.17 mmol) and toluene (30 mL) were placed into a flask. Azotropic distillation was performed to remove water from the MPEG and toluene. The toluene was then removed via distillation under a dry nitrogen stream. CH₂Cl₂ (50 mL) was added to the MPEG with CL (9.08 g, 79.53 mmol) and CL-OBn (0.92 g, 4.19 mmol), followed by the addition of a 1.0 M solution of HCl in diethyl ether (8.33 mL, 5.00 mmol) at room temperature. After 24 h, the mixture was poured into a mixture of n-hexane and EA (v/v = 40/10) to precipitate the copolymer. The precipitated copolymers were obtained from the supernatant by decantation, dissolved in CH₂Cl₂, and then filtered. The resulting copolymer solution was concentrated by rotary evaporation and dried under vacuum, yielding a colorless copolymer. In the same way, MC-(OBn)₅, MC-(OBn)₁₀, and MC-(OBn)₁₃ with respective molecular weights of 2400 g/mol, and MC-(OBn)₅ with respective molecular weights of 10,000, 50,000, and 200,000 g/mol were prepared. The molecular weights and ratios of the MC-OBn segments in the copolymers were determined by comparing the intensity of methylene carbon of poly (CL) and the phenyl carbon of poly (CL-OBn).

2.6. Synthesis of MPEG-b-[poly(ε-caprolactone)-ran-poly(4-hydroxy-ε-caprolactone)] (MC-OH)
MC-(OBn)₅ (9.40 g, 3.65 mmol) was dissolved in anhydrous THF (150 mL). Ten percent (w/w: 3 g) of Pd/C (pillow, 10 wt% (wet basis)) on activated carbon (50% water w/w, Degussa type E101 NE/W) was added to the MC-(OBn)₅ solution at room temperature under nitrogen, and the mixture was stirred under a hydrogen atmosphere for 5 h. The reaction solution was filtered through celite. The organic phase was concentrated by using a rotary evaporator and dried under vacuum to obtain a colorless copolymer (MC-(OH)₅).

2.7. Determination of sol-to-gel phase-transition times via tilting experiment
To determine the gelation time of the MC, MC-OBn, and MC-OH copolymer solutions, the copolymers (375 mg/1.5 mL) were dissolved in DW at concentrations of 20 wt% in 4 mL vials at 80 °C. The MC, MC-OBn, and MC-OH copolymer solutions, the copolymers (375 mg/1.5 mL) were dissolved in DW at concentrations of 20 wt% in 4 mL vials at 80 °C. The MC, MC-OBn, and MC-OH copolymer solutions were maintained at 37 °C. All glasses were heated under vacuum and dried under a Peltier temperature-controlled system for the bottom plates and a 25.0 mm stainless-steel parallel-plate measuring system. All measurements were conducted by using parallel-plate with a gap length of 0.3 mm. The oscillatory stress curves of storage (G') and loss modulus (G'″) were measured at 1 Hz oscillating frequency and...
amplitude gamma = 0.01—1000% at 37 °C. The storage modulus $G'$ and loss modulus $G''$ were also measured as a function of time at a frequency of 1 Hz under a 1.0% strain level at 37 °C. The gelation time was determined at the crossover point of the $G'$ and $G''$ curves. All rheological properties (viscosity, $G'$, $G''$ and phase angle (tan δ)) were analyzed with the instrument software (Rheoplus/32, version V3.21, Anton Paar).

2.9. SEM measurement

The MC, MC-OBn, and MC-OH copolymer solutions (20 wt %) was prepared and stored at 4 °C for 48 h. Then, the homogeneous opaque emulsions in the vials were incubated at 37 °C for 30 min. The prepared opaque emulsions were carefully poured on metal stubs for SEM. To minimize creation or reinforcing of the certain interaction between MC, MC-OBn, and MC-OH, the prepared opaque emulsions quickly placed in liquid nitrogen and followed by freeze-drying at -75 °C. The dried MC, MC-OBn, and MC-OH opaque emulsions on the metal stub was coated with a thin layer of gold using a plasma sputtering apparatus (Cressington 108 Auto Sputter Coater, Ted Pella Inc.; Redding, CA, USA) under an argon atmosphere.

3. Results and discussion

3.1. Preparation of 4-benzyloxy-cyclohexanone (CL-OBn)

The preparation of CL-OBn was carried out according to Fig. 1 (Scheme 1). The ketone group of 1,4-cyclohexanedione mono-ethylen acetal was reduced to a hydroxyl group by using NaBH4 and then modified by introducing a benzyl group. Subsequent hydrolysis of the acetal group gave 4-benzyloxy-cyclohexanone (3). Finally, CL-OBn was obtained by reduction of the ketone group site of 3 with mCPBA. The total yield after the four steps was almost 45%. The structure of CL-OBn was confirmed by $^1$H- and $^{13}$C-NMR and elemental analysis (see Experimental section).

3.2. Preparation of MC, MC-OBn, and MC-OH diblock copolymers

We previously reported that MC diblock copolymers were obtained by ROP of the CL monomer using the terminal alcohol of MPEG as the initiator in the presence of HCl Et$_2$O [18]. The polymers with molecular weights of less than 2000 g/mol and over 3000 g/mol were completely soluble and only partially soluble in water, thus showing no phase transition [20]. Thus, in this study, we designed diblock copolymers with an MPEG chain with MW = 750 g/mol and polyester segments with different ratios of CL/CL-OBn with the same molecular weight (2400 g/mol); segments with different molecular weights having the same CL/CL-OBn (97/3) ratio were also employed.

Firstly, MC-OBn was synthesized by ROP using varying ratios of CL and CL-OBn by employing MPEG in the presence of HCl Et$_2$O at room temperature (Fig. 1, Scheme 2). The results of synthesis of the MC and MC-OBn diblock copolymers under different conditions are summarized in Table 1.

Both CL and CL-OBn showed complete conversion. Additionally, the colorless MC-OBn diblock copolymers were obtained in almost quantitative yield. All MC-OBn diblock copolymers exhibited a monomodal GPC curve without any dead traces (Supplementary Materials Fig. S1). The polydispersity indexes of MC-OBn diblock copolymers increased slightly with an increase in the CL-OBn content, and the polydispersity index of MC-OBn$_{73}$ reached about 1.17, which is still narrow when compared to the PDI of the initial MPEG initiator of 1.06. Collectively, this showed that ROP of CL and CL-OBn might occur at almost the same rate in the presence of HCl Et$_2$O.

$^{1}$H- and $^{13}$C-NMR analyses of the MC-OBn diblock copolymers showed the characteristic peaks of MPEG, poly (CL), and poly (CL-OBn) (Fig. 2 and Supplementary Materials Fig. S2). $^{1}$H-NMR signals of the methoxy, benzyl, and phenyl protons 1, 13, and 14 were respectively observed at δ = 3.29, 4.54, and 7.39 ppm. $^{13}$C-NMR signals of methylene carbon 3 of PCL and the phenyl carbon 14 segment were observed at δ = 34.5 and 127.9 ppm, respectively. The ratios of the poly (CL) and poly (CL-OBn) segments in the MC-OBn copolymers were determined from the proton- or carbon-integration ratios from the $^{1}$H- and $^{13}$C-NMR spectra. The calculated segment ratios of poly (CL) and poly (CL-OBn) in MC-OBn agreed well with the expected ratios. This indicated that the ROP procedure used herein yielded quantitatively targeted, MC-OBn diblock copolymers with controllable benzyl pendant-group contents of 3–13 mol%.

The change in the molecular weight with variation of the feed ratio of CL/CL-OBn (97/3, mol%) to MPEG as an initiator was examined. Fig. 3 shows a plot of the MW versus the CL/CL-OBn to MPEG feed ratio. The molecular weights of MC-OBn of 10,000, 50,000, and 200,000 g/mol, calculated from the $^{1}$H-NMR spectra, increased almost linearly with increasing CL/CL-OBn to MPEG feed ratio. The results showed good agreement with those calculated from the CL/CL-OBn to MPEG feed ratios, while maintaining comparably narrow molecular weight distributions. This indicated that the ROP procedure used herein yielded targeted prepare benzyl pendant group–functionalized amphiphilic diblock copolymers with high molecular weights by the metal-free method.

For the ensuing modification, MC-OH was obtained by deprotection of the benzyl moiety in the MC-OBn diblock copolymers with benzyl pendant-group contents of 3–13 mol% by using Pd/C (Fig. 1, Scheme 2). The signal of the phenyl group of the MC-OBn diblock copolymers at 7.2–7.4 ppm (assigned to the phenyl groups) completely disappeared. Additionally, the single-modal GPC charts indicated that the deprotection reaction did not induce any side reaction on the MC segments. These results demonstrate that the deprotection successfully provided targeted MC-OH with hydroxyl pendant-group contents of 3–13 mol% (Fig. 4).

Collectively, these results demonstrate that this ROP approach successfully MC-OBn and MC-OH with controllable molecular weights and benzyl and hydroxyl pendant-group contents.

3.3. Phase transition of the MC, MC-OBn, and MC-OH diblock copolymer suspensions

Aqueous solutions of MC-OBn and MC-OH with a functional group content of 3–13 mol% were prepared as homogeneous white opaque emulsions. As a control, the MC suspension also formed opaque emulsions (Fig. 5).

The morphologies of the MC, MC-OBn, and MC-OH opaque emulsions were monitored by SEM (Supplementary Materials Fig. S3). MC-OBn and MC-OH opaque emulsions showed SEM images with water-swelled mesomorphic shape. We conjecture that MC, MC-OBn, and MC-OH in aqueous solution were firstly formed into micelles of opaque emulsions, then aggregation of micelles and finally hydrophobic segment aggregated hydrogels. In addition, we conjecture that the pendant functional groups on MC-OBn and MC-OH hydrogels altered the packing or aggregation in the micelle formation and then affect the occurrence of the macroscopic gelation of mesomorphic shape through stabilization or destabilization of intra- and inter-molecular hydrophobic aggregation.

Next, the phase transition and gelation time were examined by visual inspection and rheological measurement of the gelation with variation of the temperature (Fig. 5). Most of the diblock copolymer
Fig. 1. Synthesis of 4-benzyloxy-ε-caprolactone (CL-OBn) (Scheme 1) and preparation of MC, MC-OBn, and MC-OH diblock copolymers by metal-free method (Scheme 2).

Table 1
Synthesis of MC, MC-OBn, MC-OH diblock copolymers with 2400 g/mol and MC-OBn with 10,000, 50,000 and 200,000 g/mol.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>(CL)/[fCL] molar ratio in feed (%)</th>
<th>(CL)/[fCL] molar ratio in feed (%)</th>
<th>M_n, NMR</th>
<th>Yield (%)</th>
<th>M_w/M_n</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC</td>
<td>100/0</td>
<td>100/0</td>
<td>750-2420</td>
<td>99</td>
<td>1.13</td>
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<tr>
<td>MC-(OBn)_3</td>
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<td>97.0/3.0</td>
<td>750-2570</td>
<td>97</td>
<td>1.14</td>
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<td>95.4/4.6</td>
<td>750-2580</td>
<td>95</td>
<td>1.14</td>
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<tr>
<td>MC-(OBn)</td>
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<td>91.0/9.0</td>
<td>750-2420</td>
<td>98</td>
<td>1.14</td>
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<td>87.1/12.9</td>
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<td>1.17</td>
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<tr>
<td>MC-(OBn)</td>
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<td>97.1/2.9</td>
<td>750-231,500</td>
<td>90</td>
<td>1.58</td>
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</table>

a Determine from ^1^H NMR spectra. MPEG: 750 g/mol.
b Measured by means of gel-permeation chromatography (based on standard polystyrene).
c PDI of MPEG: 1.06.
In the tilting experiment, MC exhibited distinct sol-to-gel phase transitions below 15 s at 37 °C. MC-OBn with 3, 5, and 10 mol% benzyl groups also exhibited sol-to-gel phase transitions at 40, 60, and 120 s, respectively. However, MC-OBn with 13 mol% benzyl groups did not show sol-to-gel phase transitions. MC exhibited larger $G'$ value than $G''$ value from the beginning time, indicating hydrogel phase. MC-OBn with 3, 5, 10 and 13 mol% benzyl groups exhibited a crossover point between $G'$ and $G''$ at ~5, ~20, and ~30 s, respectively (Supplementary Materials Fig. S4). A crossover point between $G'$ and $G''$ indicated the gelation time through distinct sol-to-gel phase transitions. It is clear that the gelation time of MC-OBn increased with increasing benzyl groups.

The gelation times observed in rheometer experiment was lower than those of tilting experiment. The discrepancy in the gelation time was probably due to the amount of hydrogel to perform the tilting and rheometer experiment (see experiment section). This may induce a difference in the thermal responsibility of hydrogel [48,49].

MC-OH with a hydroxyl group also showed similar sol-to-gel phase transitions with MC-OBn, but the gelation time by visual inspection and rheological measurement was short compared with that of MC-OBn. This is due to the reduced prevention of the hydrophobic aggregation between the MC segments by the hydroxyl group.

These results indicate that the phase transition and gelation time were affected by the variation of the content of benzyl and
3.4. Rheological properties of the diblock copolymer suspensions

Rheological analysis of the diblock copolymer suspensions was performed to examine the phase transition behavior in the temperature range of 10 °C to 60 °C (Fig. 6). The MC, MC-OBn, and MC-OH diblock copolymer suspensions exhibited a viscosity of almost 1 cP at low temperatures, indicating a homogeneous suspension phase due to complete dissolution of the diblock copolymers in water.

With increasing temperature, the diblock copolymer suspensions exhibited an increase in the viscosity and reached a maximum viscosity, indicating gel-like behavior. The viscosities and onset temperatures of each MC-OBn and MC-OH copolymer suspension depended on the presence of either benzyl or hydroxyl pendant groups, as well as the amount of benzyl and hydroxyl pendant groups in the block copolymer structure.

The onset temperature (about 33 °C) was similar for MC-OBn with 3 and 5 mol% benzyl groups. The onset temperature moved to 30 °C for MC-OBn with 10 mol% benzyl groups and could not be detected for the 13 mol% sample. The maximum viscosity of MC-OBn was 2.56 × 10^6 cP when the content of benzyl groups was 3 mol% and decreased to 1.86 × 10^6 cP at 5 mol%, 1.44 × 10^6 cP at 10 mol%, and 1.4 × 10^5 cP at 13 mol%, indicating a decrease as the benzyl group content increased. This implies that the benzyl pendant groups on MC can interrupt hydrophobic aggregation between the MC segments, although the benzyl group possesses hydrophobic character.

MC-OH with 3 and 5 mol% also exhibited similar onset temperatures of 33–35 °C, whereas the onset temperature could not be detected for MC-OH with 13 mol% hydroxyl groups. The maximum viscosity of MC-OH was also reduced from 2.09 × 10^6 cP at 3 mol% to 1.5 × 10^6 cP at 13 mol% as the hydroxyl group content increased. The maximum viscosity of MC-OH was slightly higher than the corresponding values for MC-OBn. This result indicated that the

### Table 2

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Phase Transition a</th>
<th>Gelation time b (s)</th>
<th>Gelation time c (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC</td>
<td>O</td>
<td>-15</td>
<td>&lt;1</td>
</tr>
<tr>
<td>MC-(OBn)3</td>
<td>O</td>
<td>-40</td>
<td>-5</td>
</tr>
<tr>
<td>MC-(OH)3</td>
<td>O</td>
<td>-20</td>
<td>&lt;1</td>
</tr>
<tr>
<td>MC-(OBn)5</td>
<td>O</td>
<td>-60</td>
<td>-20</td>
</tr>
<tr>
<td>MC-(OH)5</td>
<td>O</td>
<td>-45</td>
<td>-5</td>
</tr>
<tr>
<td>MC-(OBn)10</td>
<td>O</td>
<td>-120</td>
<td>-30</td>
</tr>
<tr>
<td>MC-(OH)10</td>
<td>O</td>
<td>-60</td>
<td>-18</td>
</tr>
<tr>
<td>MC-(OBn)13</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MC-(OH)13</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

V a Sol-to-gel phase transition at 37 °C.

b Measured by tilting experiment (O: gel formation at 37 °C, X: sol maintenance at 37 °C).

c Measured by rheometer.
hydroxyl groups on MC slightly induced gelation via intra and intermolecular hydrogen bonding interactions, although hydrogen bonding in temperature-responsive hydrogels is a minor interaction due to strong competition with excess water under the aqueous condition.

In oscillatory stress measurements, the storage and loss moduli of MC-OBn and MC-OH hydrogels were measured. The $G'$ and $G''$ of the hydrogels decreased as the benzyl group or hydroxyl group content increased (Supplementary Materials Fig. S5). Moreover, the damping factor (defined as the tan $\delta$ values calculated from $G''/G'$) of each MC-OBn and MC-OH increased as the benzyl group or hydroxyl group content increased, indicating the decreasing gel-like feature. These results indicated that stiff, hydrogel-like features of MC-OBn and MC-OH had significantly affected according to the pendant group contents.

The concentration of MC-OBn and MC-OH hydrogels can induce the different thermal responsibilities as a function of temperature. Thus, the phase transition of the different concentrations of MC-OBn and MC-OH suspensions was measured from 10°C to 60°C (Fig. 7). All concentrations of MC-OBn, and MC-OH diblock copolymer suspensions exhibited a viscosity of almost 1 cP at low temperatures. At intermediate temperature, the maximum viscosities of MC-(OBn)$_3$ decreased from 1.8 × 10$^3$ cP at 20 wt%, to $9.9 \times 10^2$ cP at 18 wt% and $4.8 \times 10^2$ cP at 15 wt% as the concentration of MC-(OBn)$_3$ decreased. No viscosity was observed at 12 wt% concentration of MC-(OBn)$_3$, exhibited no viscosity. Additionally, MC-(OH)$_3$ and MC-(OH)$_5$ exhibited almost similar viscosity changes as the concentration of MC-(OH)$_3$ and MC-(OH)$_5$ decreased. The maximum viscosities of MC-(OH)$_3$ and MC-(OH)$_5$ exhibited a slightly higher than those of MC-(OBn)$_3$ and MC-(OBn)$_5$. These results implied that increasing the hydroxyl pendant group content of MC-OH can enhance the hydrophilic properties of the MC segments in the suspension.

The thermal reversibility was confirmed by conducting heating and cooling cycle for MC-(OBn)$_3$ and MC-(OH)$_3$ (Supplementary Materials Fig. S6). The viscosity in cooling cycle of MC-(OBn)$_3$ and MC-(OH)$_3$ after heating exhibited relatively larger maximum one at the low temperature. This result indicated the thermal reversibility of MC-(OBn)$_3$ and MC-(OH)$_3$, although the viscosity change curves did not completely overlapped in heating and cooling cycle.

Taken together, the sol-to-gel phase transition of the MC-OBn and MC-OH diblock copolymers may be affected by the type and concentrations of the pendant groups, or suspension concentration of the MC-OBn and MC-OH diblock copolymers because the pendant groups on MC segments might stabilize or destabilize hydrophobic aggregation between the MC segments via intra- and inter-molecular MC hydrophobic aggregation in aqueous solutions.
3.5. Crystalline properties of MC, MC-OBn, and MC-OH diblock copolymer suspensions

To understand the hydrophobic aggregation of the MC segments in aqueous solutions, the crystalline properties of the MC, MC-OBn, and MC-OH diblock copolymer suspensions were measured (Table 3). The enthalpy of crystallization was determined by DSC analyses of the diblock copolymer suspensions.

The crystallization enthalpy of the MC diblock copolymer suspension were quantified as 11.7 J/g. The crystallization enthalpy of the MC-OBn diblock copolymer suspensions decreased from 9.5 J/g at 3 mol% benzyl pendant groups, to 5.7 J/g at 5 mol% benzyl pendant groups, and to 2.7 J/g at 10 mol% benzyl pendant groups. The crystallization enthalpy of MC-OBn with a benzyl pendant group content of 13 mol% approached zero. These results strongly indicated that the benzyl pendant group on the MC segment prohibited hydrophobic aggregation between the MC segments.

The MC-OBn diblock copolymer suspensions with 3–13 mol% of hydroxyl pendant groups on the MC segment exhibited slightly higher values of crystallization enthalpy than the MC-OBn diblock copolymer suspensions. The MC-OBn diblock copolymer suspensions with 3 mol% of hydroxyl pendant groups had respective values of crystallization enthalpy of 11 J/g, which are similar to those of MC, probably because MC-OH with a low hydroxyl content of 3 mol% prevented hydrophobic aggregation to a lesser degree. The crystallization enthalpy of the MC-OH diblock copolymer suspensions decreased from 11 J/g to 5.9 J/g with an increase in the hydroxyl pendant group content. These results implied that increasing the hydroxyl pendant group content can enhance the hydrophilic properties of the MC segments in the suspension. Moreover, the benzyl pendant group on the MC segment interfered with hydrophobic aggregation between the hydrophobic MC segments to a greater extent than the hydroxyl pendant group.

Collectively, these results strongly support the idea that crystallinity in the MC-OBn and MC-OH diblock copolymer suspensions stabilizes hydrophobic aggregation via intra- and inter-molecular interactions of the MC segments.

4. Conclusions

In conclusion, the ROP of CL and CL-OBn by the metal-free method successfully provided MC-OBn diblock copolymers with segment ratios and molecular weights that were close to the theoretical values. The MC-OH diblock copolymers were also obtained by a deprotection reaction. MC-OBn and MC-OH acted as temperature-responsive hydrogels that were influenced by the type and content of pendant groups. The pendant groups on the MC segment weakened and/or prohibited hydrophobic aggregation between the MC segments. We believe that the temperature-responsive MC-OBn and MC-OH hydrogels can be utilized for practical biomedical applications.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.polymer.2018.01.001.

References
