Evaluation of Enzymatic Degradation of Gel and Colloid Formed by Poly(trimethylene carbonate) Grafted Copolymer

Kyohei Nitta, Junji Watanabe, and Yoshiyuki Ikeda

Department of Chemistry of Functional Molecules, Faculty of Science and Engineering, Konan University

Fax: +81-78-435-2539, E-mail: junjiknd@konan-u.ac.jp, yikeda@konan-u.ac.jp

Amphiphilic graft copolymers were synthesized using *N*-hydroxyethyl acrylamide (HEAA) as the hydrophilic main chain and poly(trimethylene carbonate) (PTMC) as the hydrophobic side chain. This amphiphilic poly(HEAA-*graft*-PTMC) (PHT) copolymers self-aggregated because of hydrogen bonds and hydrophobic interaction. The PTMC side chain was incorporated into the hydroxyl group on HEAA via ring-opening polymerization of a trimethylene carbonate monomer using an organic catalyst at room temperature. Enzymatic degradation of PHT was performed using the polymer gel and colloid samples in the presence of lipase in phosphate buffered saline (PBS) solution (pH 7.1) at 37°C. The degradation behavior of this polymer colloid was observed by dynamic light scattering. The surface morphology of PHT was evaluated by scanning electron microscopy. The PHT copolymer was slightly degraded by lipase in PBS, indicating its potential as a carrier of drug delivery systems.

Key words: amphiphilic graft copolymer, *N*-hydroxyethyl acrylamide, poly(trimethylene carbonate), lipase, colloid gel

1. Introduction

Amphiphilic copolymers possess both hydrophobic and hydrophilic properties. This enables their application in various fields as they can form a variety of micellar structures in selected solvents. In the field of biomaterials, amphiphilic copolymers designed using biocompatible and biodegradable polymers are widely investigated for use as carriers of drug delivery systems (DDS). In this study, we focused on the hydrophobic polymer poly(trimethylene carbonate) (PTMC). As a biodegradable polymer, PTMC is a well-known biomaterial and is easily prepared from the cyclic monomer trimethylene carbonate (TMC). TMC is polymerized from the hydroxyl group by a conventional ring-opening polymerization (ROP) technique using a metal-free catalyst [1–3]. The properties of PTMC include *in vivo* degradability without acidic degradation products, good biocompatibility, excellent mechanical properties with a low glass transition temperature, and an easy and precise method of synthesis [3–8]. Therefore, PTMC is considered to be a promising biomaterial.

We designed a novel graft copolymer with a main chain consisting of poly(*N*-hydroxyethyl acrylamide) (poly(HEAA)) and a side chain consisting of a PTMC segment with controlled chain length. Amphiphilic graft polymers have the advantage that their hydrophilic–hydrophobic balance can be altered by controlling their composition ratio and the chain length of their hydrophobic segment. In a previously published paper, we reported the synthesis of a *N*-hydroxyethyl acrylamide (HEAA)–PTMC macromonomer with an oligo hydrophobic segment and of

poly(HEAA-*graft*-PTMC) (PHT) by the macromonomer method [8, 9]. In an aqueous solution, these PHT copolymers formed a hydrogel as a result of the molecular forces of both hydrogen bonds and hydrophobic interactions, and the PTMC hydrophobic domain of this aggregate was able to incorporate a fluorescence probe. In our previous study, we performed fluorescence analysis of the PHT copolymers PH₉₉T₁₁, PH₉₂T₁₂, and PH₉₉T₄₈. Here, the sample code PH₉₉T₁₁ indicates that the HEAA monomer composition ratio was 99 mol% and the polymerization degree (DP) of the PTMC segment was 11.

In the field of DDS, a polymer gel or polymer micelle that is used as a drug carrier must degrade *in vivo* and release the drug for a given amount of time. Lipase plays a crucial part in the biodegradation of PTMC *in vivo*. Watanabe *et al.* have reported that an amphiphilic block copolymer with a high-molecular-weight PTMC segment was degraded by lipase within 30 h. In this study, we observed the degradation behavior of PTMC *in vitro* using lipase in a phosphate buffered saline (PBS) solution at 37°C for a given period of time [10, 11]. We evaluated the degradation behaviors in terms of the swelling ratio and the particle size distribution of the polymer colloid. The moderate degradation rates of the copolymers depended on both the composition ratios of the HEAA–PTMC hydrophobic macromonomer and the chain length of the PTMC.

2. Experimental

2.1. Materials

Table 1. Composition and molecular weight of poly(HEAA-graft-PTMC).

Sample code	Composition ratio (^1H NMR)		Polymerization degree of PTMC (^1H NMR)	GPC		
	HEAA (mol%)	HEAA-PTMC (mol%)		M_n	M_w	M_w/M_n
PH ₉ T ₁₁	99.0	1.0	10.6	28000	109000	3.84
PH ₉ T ₁₂	92.4	7.6	11.9	82000	405000	5.54
PH ₉ T ₄₈	99.3	0.7	47.5	77000	207000	2.67

For the synthesis of the HEAA-PTMC macromonomer, the hydrophilic monomer HEAA was gifted by Kohjin Co., Ltd., Tokyo, Japan. The cyclic monomer TMC was obtained from Boehringer Ingelheim Pharma GmbH, Ingelheim, Germany. The organic catalyst 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; Kanto Chemical Co., Ltd., Tokyo, Japan) was used for the activation of the hydroxyl group in HEAA. The terminating agent benzoic acid was obtained from Wako Pure Chemical Industries, Osaka, Japan. The radical polymerization of PHT was performed by using 2,2'-azobis(isobutyronitrile) (AIBN; Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) as the initiator. Lipase from *Phycomyces nitens* (activity: 100–200 units/mg) for the hydrogel degradation test was purchased from Wako Pure Chemical Industries, Osaka, Japan. The organic solvents dichloromethane and *N,N*-dimethylformamide (DMF) were used as received (Wako Pure Chemical Industries, Osaka, Japan).

2.2. Characterization

Chemical structures, the DP of the PTMC segment, and the composition ratios of the HEAA monomer and HEAA-PTMC macromonomer were determined by ^1H NMR spectra (Unity INOVA AS500 MHz). The samples were dissolved in deuterated chloroform (CDCl_3) or dimethyl sulfoxide ($\text{DMSO}-d_6$) in the presence of tetramethylsilane as an internal standard. To determine molecular weight and molecular weight distribution, gel permeation chromatography (GPC; Shodex GPC, Showa Denko, Tokyo, Japan) was performed using a DMF solvent with a lithium bromide additive at a concentration of 10 mmol/L. Field emission scanning electron microscopy (SEM; JSM-6340FB, JEOL, Tokyo, Japan) was used to observe the surface morphology of the freeze-dried PHT. The size distribution of the polymer colloid was determined by particle size distribution measurement (Zetasizer Nano ZS, Malvern Instruments Ltd., UK).

2.3. Preparation of Amphiphilic Graft Copolymers with PTMC Segments

Amphiphilic graft copolymers with PTMC segments were prepared according to the procedure reported previously [8, 9]. First, the HEAA-PTMC macromonomer was synthesized by typical ROP of TMC monomer. ^1H NMR spectrum (500 MHz, CDCl_3) was assigned as follows; δ (ppm): 2.1 (m, 2H, $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$), 3.6 (q, 2H, $-\text{CH}_2-\text{CH}_2-\text{O}-$), 3.7 (t, 2H, $-\text{NH}-\text{CH}_2-\text{CH}_2-$), 4.2 (t, 4H, $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$), 5.6 (d, 1H, $\text{H}-\text{CH}=\text{CH}-$), 6.1 (q, 1H, $\text{CH}_2=\text{CH}-\text{CO}-$), 6.2 (br, 1H, $-\text{CO}-\text{NH}-\text{CH}_2-$), 6.3 (d, 1H, $\text{H}-\text{CH}=\text{CH}-$).

Next, conventional radical polymerization was carried out using AIBN as the initiator, HEAA

Figure 1. Structure of poly(HEAA-graft-PTMC).

monomer, and HEAA-PTMC macromonomer. ^1H NMR spectrum (500 MHz, $\text{DMSO}-d_6$) was assigned as follows; δ (ppm): 1.1–1.6 (br, 3H, $-\text{CH}_2-\text{CH}-\text{CO}-$), 1.9 (m, 2H, $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$), 3.4–3.5 (br, 4H, $-\text{NH}-\text{CH}_2-\text{CH}_2-$), 4.1 (t, 4H, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{O}-$), 7.2–7.9 (br, 1H, $-\text{CO}-\text{NH}-\text{CH}_2-$). The molecular weight was measured by GPC, and the surface morphology was observed by SEM.

2.4. Enzymatic Degradation of Polymer Gel by Lipase

Enzymatic degradation of the PHT copolymers by lipase in PBS solution was evaluated by measuring the weight of the swelled hydrogel and the particle size of the polymer colloid. The swelled hydrogel was prepared according to the following procedure. PHT copolymer sample was dissolved in PBS solution at a concentration of 1 mg/mL in a nylon mesh bag, and allowed to soak in the PBS solution for 1 h. After the weight of this hydrogel was measured, it was added to a lipase PBS solution with a concentration of 1 w/v%. The weight of the hydrogel was measured for a given period. The swelling ratio was calculated using the following equation:

$$\text{Swelling ratio (\%)} = \frac{W_s - W_0}{W_0} \times 100$$

where W_s is the weight of the swelled polymer gel and W_0 is the weight of the dry polymer gel. The particle size distribution of the polymer colloid in PBS lipase solution was measured using the dynamic light scattering (DLS) technique for a given period. The temperature was maintained at 37°C during the enzymatic degradation.

3. Results and Discussion

3.1. Synthesis of Amphiphilic Graft Copolymer with PTMC Segments

Precise ROP of the cyclic monomer TMC was performed by adjusting the concentration of the DBU

Figure 2. Scanning electron microscopy images of poly(HEAA) homopolymer and poly(HEAA-*graft*-PTMC). (a) Poly(HEAA), (b) PH₉₉T₁₁, (c) PH₉₉T₁₂, and (d) PH₉₉T₄₈. Scale bar: 10 μ m.

catalyst. We obtained HEAA-PTMC macromonomer, in which the chain length of PTMC was controlled. The copolymer PHT, which was synthesized by radical polymerization, possessed the target composition and side chain length (Table 1 and Figure 1). Figure 2 shows the surface morphologies of the copolymer observed using SEM. The poly(HEAA) image (Figure 2(a)) shows the surface pores created by water evaporation. These pores were of approximately the same size ($\approx 2 \mu$ m). The images of PH₉₉T₁₁, PH₉₉T₁₂, and PH₉₉T₄₈ (Figure 2 (b)–(d)) show a similar bundle structure. PH₉₉T₁₁ and PH₉₉T₁₂ possessed a DP of PTMC of approximately 10 and formed irregular spheres. PH₉₉T₁₁, which has a macromonomer composition ratio of 1 mol% and a DP of PTMC of 11, formed a unidirectional bundle. PH₉₉T₁₂, with a high composition ratio of macromonomer, formed a random bundle structure. These morphologies were depending on the number of physical cross-linking by both hydrogen bond of HEAA and hydrophobic interaction of PTMC. The surface structure of PH₉₉T₄₈ was fibrous with a longer chain length than those of PH₉₉T₁₁ and PH₉₉T₁₂. Therefore, polymer surface morphology of the PHT copolymers could be controlled by the alteration of chain length and composition ratio.

3.2. Degradation Behavior of Polymer Hydrogel and Polymer Colloid by Lipase

We investigated the swelled gel weight to observe the degradation behavior by lipase (Figure 3). PH₉₉T₁₁ data are not shown due to its fast hydrolysis in PBS solution (within 1 h) before the addition of the PBS lipase solution. PH₉₉T₁₂ maintained a constant swelling ratio (300%) in PBS lipase solution within 6 h. Figure 3(a) shows that significant enzymatic degradation behavior by lipase was not observed over a short period

because PH₉₉T₁₂, with its higher HEAA-PTMC macromonomer composition ratio, has a complex network structure stabilized by a large number of cross-linkages. PH₉₉T₄₈ showed slightly faster degradation in the presence of lipase than in its absence. However, PH₉₉T₄₈ displayed a “broken-up” appearance and the small species leaked from the nylon mesh bag. Therefore, we investigated the polymer colloid sizes in the presence of lipase in PBS solution by DLS. Figure 4 shows that the particle size distribution in PBS solution are almost a monodispersed distribution, with the exception of PH₉₉T₁₂. These size distributions changed with the addition of 1 w/v% lipase and a large size distribution, ranging from 10 to 1000 nm, was observed. All samples were degraded for a given period. PH₉₉T₁₁ began to degrade soon after the addition of lipase. PH₉₉T₁₂ and PH₉₉T₄₈ were slightly degraded for 30 h. The size distribution shifted to the low particle-size range. A similar size distribution was also observed for the poly(HEAA) polymer. We hypothesized that PH₉₉T₁₂ and PH₉₉T₄₈ were not dissociated by water and PBS solution because they have more TMC units than PH₉₉T₁₁. This result indicated that microscopic degradation behavior was observed by DLS. The degradation experiment revealed that the copolymer with PTMC of higher molecular weight than our synthesized copolymer with PTMC oligo segment was easy to degrade by lipase for short period [10–12]. We concluded that the copolymer with PTMC of a low molecular weight was slightly degraded for a low lipase activity in a short period and it was formed intense bundle structure as shown in Figure 2(c) [13]. We hypothesized that the oligo PTMC segment in PHT could not interact well with the lipase macromolecule.

Figure 3. Degradation behavior of poly(HEAA-graft-PTMC) (a) PH₉₂T₁₂ and (b) PH₉₉T₄₈ by lipase. The change in swelling ratio was plotted as a function of degradation time. Closed symbol indicates in the presence of lipase in PBS solution at 37°C. Open symbol indicates without lipase in PBS solution at 37°C.

Figure 4. Change in size distribution intensity of polymer colloid by addition of lipase was determined by DLS measurement. (a) Poly(HEAA), (b) PH₉₉T₁₁, (c) PH₉₂T₁₂, and (d) PH₉₉T₄₈.

4. Conclusion

We obtained target PHT copolymers via a two-step synthesis route using conventional ROP and radical polymerization. The properties of the PHT copolymers were investigated in terms of ¹H NMR, GPC, SEM, swelling ratio, and DLS. In the SEM images, the surface morphology of the PHT was observed to display a unique network structure. Lipase caused PHT to display slight degradation behavior over a short period. PHT copolymers with an oligo PTMC segment are suited for the enhancement of retention in DDS. We confirmed that PHT copolymers have potential as hydrophobic drug delivery vehicles.

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6. References

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