Temperature-controlled erosion of poly(\(N\)-isopropylacrylamide)-based hydrogels crosslinked by methacrylate-introduced hydrolyzable polyrotaxane

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Abstract

Biodegradable hydrogels for temperature-controlled erosion were prepared by co-crosslinking \(N\)-isopropylacrylamide (NIPAAm) and methacrylate (MA)-introduced polyrotaxane (PRX) in which many \(\alpha\)-cyclodextrins (\(\alpha\)-CDs) are threaded onto a poly(ethylene glycol) (PEG) chain capped with bulky end-groups via ester linkages. The amount of MA attached to hydroxyl group of \(\alpha\)-CDs in PRX could be varied by the feed ratio of GMA and PRX. The prepared hydrogels were transparent below lower critical solution temperature (LCST) of PNIPAAm matrix (32°C). By elevating temperature above the LCST, water contents were slightly decreased, and the hydrogels became opaque. Elevating temperature in an aqueous condition above the LCST led to dehydration of the PNIPAAm matrix, which accompanies \(\alpha\)-CD sliding to expose the ester linkage to the medium and enhances erosion of the hydrogels.

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1. Introduction

Biodegradable polymers have been used as scaffolds for tissue engineering and implantable drug delivery. Scaffolds should be made of biodegradable polymers in which the degradation rate is desirable to be synchronized with the extent of tissue regeneration and drug release. However, it is difficult to control the degradation time in the highly water friendly conditions due to uncontrolled water intrusion to hydrolyzable linkages. We have studied hydrolyzable polyrotaxanes (PRXs), in which many \(\alpha\)-cyclodextrins (\(\alpha\)-CDs) are threaded onto a poly (ethylene glycol) (PEG) chain capped with bulky end-groups via ester linkages [1]. These hydrolyzable PRXs were utilized as a cross-linker to prepare PEG hydrogels for biomedical applications [2]. In this system, PEG-bisamine was bound with hydroxyl groups of \(\alpha\)-CDs threaded onto another PEG chain end-capped via ester linkages: mechanical locking between \(\alpha\)-CDs and PEG chain is utilized as a cross-link point that is mobile along the threading PEG chain. Controlling inclusion complexation between \(\alpha\)-CDs and the ester linkage by the introduced number of PEG-bisamine led to prolonged time to complete hydrogel erosion from day to month, even at extremely high water contents[3,4]. Our next concern is how to control the inclusion complexation to change the erosion time by external stimuli. Temperature is one of the most useful external stimuli that strongly influence the state of hydrogels. A representative of the temperature-responsive polymers is poly(\(N\)-isopropylacrylamide) (PNIPAAm) and its copolymers. They exhibit lower critical solution temperatures (LCSTs) in aqueous solution where their hydration-dehydration change occurs reversibly in response to small temperature change [5]. PNIPAAm can form an expanded structure below the LCST, while it can form a compact structure above the LCST with dehydration. From the characteristics of conformational change, we hypothesized that PNIPAAm hydrogels crosslinked by PRXs can modulate inclusion complexation between \(\alpha\)-CDs and the ester linkage in PRXs.
In this study, NIPAAm was co-crosslinked with a methacrylate (MA)-introduced PRX to achieve controlled erosion of hydrogels in synchronized with temperature. The hydrogels are prepared from the MA-introduced PRX and NIPAAm with different contents and evaluated the erosion behavior with the different temperatures.

2. Methods

2.1. Materials

Poly(ethylene glycol) (PEG) with the number-average molecular weight ($M_n$) of 3300 was kindly supplied from Sanyo Chemical Co. Ltd, Kyoto, Japan. $\alpha$-Cyclodextrin ($\alpha$-CD) was purchased from Bio-Research Corporation, Yokohama, Japan. Benzylloxycarbonyl L-phenylalanine (Z-L-Phe), succinic anhydride, ethylenediamine, N,N'-dicyclohexylcarbodiimide (DCC), N,N'-dimethylaminopyridine (DMAP), glycidyl methacrylate (GMA), N-isopropylacrylamide (NIPAAm) and ammonium peroxodisulfate (APS) were purchased from Wako Pure Chemical Co. Ltd, Osaka, Japan. N-Hydroxysuccinimide (HOSu) was purchased from Peptide Institute, Inc., Osaka, Japan. Dimethylsulfoxide (DMSO) was purchased from Nakarai Tesque, Inc., Kyoto, Japan and was distilled under vacuum at 78 C/12 mmHg. Other reagents were used as received without further purification. NIPAAm was recrystallized from hexane. A hydrolyzable polyrotaxane (PRX-$\alpha_{15/3.3}$ K, threading # of $\alpha$-CD: 15, $M_n$ of PEG: 3,300) was prepared according to our previous method [1,3].

2.2. Preparation of methacrylate (MA)-introduced PRX (Scheme 1)

MA groups were introduced into PRX by the addition reaction of GMA with hydroxyl groups of $\alpha$-CDs [6]. Here, the feed amounts of PRX and GMA were varied as shown in Table 1. For example, PRX (4 g, 0.14 mmol) was dissolved in DMSO (100 ml), DMAP (500 mg, 4.09 mmol) and GMA (1.44 ml, 10.9 mmol) were added to the solution, and the solution was stirred at 60 °C for 12 h. The reaction mixture was poured into excessive diethyl ether, and the resulting precipitate was dried in vacuo at room temperature. The introduction of MA to PRX was characterized by $^1$H-NMR spectroscopy using a 300 MHz FT-NMR spectrometer (Varian, Unity plus, CA).

3. Preparation of the PRX-MA/NIPAAm hydrogels (Scheme 1) and in vitro erosion

The MA-introduced PRX/NIPAAm hydrogels (PRX-MA/NIPAAm hydrogels) were prepared by radical copolymerization of the MA-introduced PRX and NIPAAm using APS as an initiator. For example, MA-introduced PRX (200 mg, 9.67 x 10^{-3} mmol), NIPAAm (62.7 mg, 0.55 mmol) and APS (20 mg, 0.09 mmol) were dissolved in DMSO (2.0 ml), and the solution was degassed by sonication under vacuum. The mixture was injected into a spacer (diameter = 15 mm, height = 2 mm) and the hydrogels were prepared by photoirradiation ($\lambda$ = 330 nm) for 5 h. The obtained hydrogels were removed from the spacer and immersed in ethanol/water (ratio = 1:1) and then distilled water at room temperature for 2 days to remove unreacted compounds. As a reference, a PEG hydrogel crosslinked by PRX was prepared according to our previous methods [3,4]. The swollen hydrogels as a function of temperature were measured after tapped with filter paper to remove excess water on the surface. Swelling ratio and water content were calculated using the following equations.

\[
\text{Swelling ratio} = \frac{W_s - W_d}{W_d} \quad (1)
\]

\[
\text{Water Content} = \frac{W_s}{W_d} \times 100 \quad (2)
\]

where $W_s$ is the weight of swollen hydrogels and $W_d$ the weight of dried hydrogels. In vitro erosion of the hydrogels...
was evaluated by weighing the residual weights of hydrogels in 0.1 M NaOHaq at 25 and 50 °C.

4. Results and discussion

4.1. Preparation of MA-introduced PRXs

Introducing MA to hydroxyl groups of α-CDs in the PRX was carried out by DMAP-catalyzed addition reaction of GMA [6]. From 1H-NMR spectra, all the peaks attributed to α-CDs (δ = 6.8 ppm: OH of C2 and C3, δ = 4.9 ppm: C1H, δ = 4.6 ppm: OH of C6, δ = 4.0–3.3 ppm: C3, C5, C6, C2 and C4H), PEG (δ = 3.6 ppm), and methacryloyl group (δ = 8.2 and 7.1 ppm: protons at double bond, δ = 1.9 ppm: methyl proton) were observed (Fig. 1). From the NMR analysis of the MA-introduced PRXs, the introduced MA per one α-CD molecule was calculated as shown in Table 1. The amount of MA could be increased with the feed amount of GMA (Fig. 2). In the view point of complete erosion of hydrogels crosslinked by the PRX, the ratio of GMA/α-CD should be below 1, because excess introducing polymer matrix to one α-CD molecule in PRX results in incomplete erosion of the hydrogels even after the ester hydrolysis of the PRX (i.e.: formation of network structure consisting of α-CDs and the polymer matrix)[3]. Therefore, PRX-GMA08 in Table 1 was chosen for preparing PRX-GMA/NIPAAm hydrogels.
4.2. Preparation of the PRX-MA/NIPAAm hydrogels

By co-crosslinking the PRX-MA08 and NIPAAm monomer, transparent hydrogels could be obtained at 25 °C (Fig. 3 left). By changing the feed weight of the PRX-MA08 and NIPAAm, two types of hydrogels (PRX-NIPAAm62-38 and PRX-NIPAAm45-55) were obtained (Table 2). Although NIPAAm content of PRX-NIPAAm62-38 was different from PRX-NIPAAm45-55, water contents below and above LCST were similar level. By elevating temperature above lower critical solution temperature (LCST) of PNIPAAm matrix (32 °C), water contents were slightly decreased, and the hydrogels became opaque (Fig. 3 right). These results suggest that the PNIPAAm matrix was dehydrated in the hydrogels above the LCST. In order to assess the effect of temperature-responsive matrix on the properties of hydrogels, a PEG hydrogel crosslinked by PRX (PRX-PEG47-53) was prepared (Table 2). Water content of PRX-PEG47-53 was not changed when temperature was elevated from 25 to 50 °C.

Table 2
Preparation of PRX-MA/NIPAAm hydrogels

| Code         | PRX-MA (wt%) | NIPAAm (wt%) | PRX-CDI (wt%) | PEG (wt%) | Water Content (% 25 °C | Water Content (% 50 °C) |
|--------------|--------------|--------------|---------------|-----------|------------------------|-------------------------|
| PRX-NIPAAm62-38 | 62           | 38           | –             | –         | 97                     | 89                      |
| PRX-NIPAAm45-55 | 45           | 55           | –             | –         | 96                     | 81                      |
| PRX-PEG47-53  | –            | –            | 47            | 53        | 78                     | 75                      |

4.3. Controlled erosion the hydrogels in response to temperature

The erosion behavior of PRX-NIPAAm62-38 and PRX-NIPAAm45-55 was monitored by weight change in an alkaline condition that can accelerate hydrolysis rate of the terminal ester linkage in the polyrotaxane crosslink [7]. Although those hydrogels were completely eroded at both 25 and 50 °C, the complete erosion times of the hydrogels were shortened by elevating temperature (Fig. 4). On the other hand, the complete erosion time of PRX-PEG47-53 under the same conditions was not changed by elevating temperature (Fig. 5). These results suggest that the temperature-responsive PNI- PAAm matrix contributes to the enhanced degradation at the elevated temperature. Since such the effect of temperature on modulating the erosion time of PRX-PEG47-53 was not observed, ester hydrolysis rate at 50 °C would be the same with at 25 °C. So, the other factors should be take into account for

Fig. 3. Temperature-responsive property of the PRX-MA/NIPAAm hydrogels.

Fig. 4. Weight change of PRX-MA/NIPAAm hydrogels in 0.1 M NaOHaq at 25 °C (blue) and 50 °C (red).

Table 2
Preparation of PRX-MA/NIPAAm hydrogels

| Code         | PRX-MA (wt%) | NIPAAm (wt%) | PRX-CDI (wt%) | PEG (wt%) | Water Content (% 25 °C | Water Content (% 50 °C) |
|--------------|--------------|--------------|---------------|-----------|------------------------|-------------------------|
| PRX-NIPAAm62-38 | 62           | 38           | –             | –         | 97                     | 89                      |
| PRX-NIPAAm45-55 | 45           | 55           | –             | –         | 96                     | 81                      |
| PRX-PEG47-53  | –            | –            | 47            | 53        | 78                     | 75                      |

a Concentration of APS in feed: 10 mg/ml DMSO.
b PRX-MA08 in Table 1 was used.
c PRX-CDI is the polyrotaxane in which hydroxyl groups of a-CDs are activated by N,N-carbonyldimidazole (see Refs. [3] and [4]).
d a,ω-di amino PEG (Mn=4,000) was used for crosslinking with the PRX-CDI (see Refs. [3] and [4]).
rationale the effect of PNIPAAm matrix. One of possible factors is inclusion and dissociation between the terminal ester linkages and \(\alpha\)-CDs in the PRX. In our previous studies, inclusion complexation between the terminal ester linkages and \(\alpha\)-CDs in the PRX was found to be enhanced by some chemical modification, which was confirmed by NMR spectroscopic analysis using an 2-aminoetanol-modified PRX [4]. In addition, cholesterol-modification of the PRX-PEG hydrogels shortened the time to complete erosion time in PBS at neutral pH condition, suggesting that hydrophobic modification of \(\alpha\)-CDs in the PRX leads to expose the terminal ester linkages to the outer medium due to sliding \(\alpha\)-CD molecules leaving from the terminals via hydrophobic interactions [7]. Considering the effect of hydrophilic and hydrophobic modification on the inclusion state-related ester hydrolysis, temperature-dependent hydration and dehydration of PNIPAAm matrix in the PRX/PNIPAAm hydrogels would alter the inclusion and dissociation of the terminal ester linkages below and above the LCST, respectively. Therefore, it is suggested that aggregation of the PNIPAAm matrix above the LCST induces the sliding \(\alpha\)-CD molecules from the terminal ester linkages, resulting in exposure of the ester linkage to the alkaline medium to enhance the erosion (Fig. 6).

5. Conclusion

Temperature-responsive and biodegradable hydrogels were prepared by co-crosslinking MA-introduced PRX and NIPAAm. Elevating temperature in an aqueous condition above the LCST led to dehydration of the PNIPAAm matrix, which accompanies \(\alpha\)-CD sliding to expose the ester linkage to the medium and enhances erosion of the hydrogels.

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