Hydrogels having tubular α-cyclodextrin structure: effect of nano-tube structure on long alkyl chain partitions

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Abstract

New hydrogels having the tubular structure of α-cyclodextrins (α-CDs) crosslinked by poly(ethylene glycol) (PEG) (MT-PEG hydrogels) were prepared by using a hydrolyzable polyrotaxane. The hydrolyzable polyrotaxane, in which many α-CDs are threaded onto a PEG chain capped by a hydrolyzable ester moiety, was used to form a tubular structure in the hydrogel. After crosslinking with another PEG chain, the ester linkage in the polyrotaxane was hydrolyzed in 1N NaOH. This led to exposing hydrophobic cavity of α-CDs. The tubular-structured hydrogel incorporated sodium dodecyl sulfate (SDS) much faster than normally crosslinked α-CDs hydrogel (α-CD-PEG hydrogel). Furthermore, partition coefficient (K) of SDS to the MT-PEG hydrogel was two times larger than the α-CD-PEG hydrogel. These results suggest that the tubular structure of α-CDs, made from a template of the polyrotaxane, is much more attractive to include SDS in the α-CD cavities. Unsaturated fatty acids (palmitoleic acid (C16:1) and oleic acid (C18:1)) were also effectively incorporated into the tubular-structured hydrogel during 6 days. The K value of C16:1 was as the same order in magnitude as C18:1. Thus, the tubular structure of α-CDs was advantageous to incorporate long alkyl chains into the hydrophobic cavity of α-CDs in aqueous conditions.

Keywords: Cyclodextrin; Tubular structure; Hydrogels; Partition; Polyrotaxane; Supramolecular structure; Fatty acids; Crosslinking; Poly(ethylene glycol)

1. Introduction

Cyclodextrins (CDs) are cyclic molecules that have the ability to encapsulate guest molecules within their hydrophobic cavities. Three well characterized and commercially available CD families are α-, β-, and γ-CDs consisting of 6, 7 and 8 glucose units, respectively. Inclusion complexation with CDs has developed the CD technology range from the agricultural, pharmaceutical [1,2], analytical and diagnostics industries to the cosmetics and food industries [3]. α-, β-, and γ-CDs have 18, 21, 24 hydroxyl groups, so that many researchers have tried to modify the groups to improve the physicochemical properties such as aqueous solubility, chemical and physical stability, selective binding, and so on [4]. Recently, CDs are used as a building block for supramolecular structures [5,6]. In the 1990s, a new type of supramolecular assembly consisting of CDs and linear polymers has been reported by Harada et al. [7–9]. They prepared a polyrotaxane, in which many α-CDs are threaded onto a poly(ethylene glycol) (PEG) capped with bulky blocking-groups [10]. Based on this supramolecular design, our group synthesized biodegradable polyrotaxanes, in which the bulky-blocking group was introduced via peptide or ester linkages [11–13]. The chemical modification of the hydroxyl groups in the polyrotaxanes has been carried out to develop the polyrotaxanes as functional materials. So far, it is possible to introduce various kinds of ligands including hydroxypropyl [12], hydroxyethylcarbamoyl [14], acetyl [15], carboxyethyl [16], biotin [17,18], valyl-lysine [19], maltosyl [20], sulfonate [21] and naphthalene [22] groups. When the hydroxyl groups are chemically modified by using bi-functional reagents, CDs in the polyrotaxanes can be intramolecularly crosslinked. In the former case, Harada et al. crosslinked adjacent CDs in the polyrotaxanes, and then hydrolyzed the terminal bulky-blocking groups to prepare a tubular conjugate, named molecular tube (MT) [23]. Our group prepared MT with very narrow molecular weight distribution and clarified thermodynamic properties of the inclusion complexion with sodium alkyl sulfonates [24, 25]. These studies suggest that the tubular structure of MT is preferable for the supramolecular interactions such as van der
Waals and hydrophobic interactions, especially with longer alkyl chains. On the other hands, chemical crosslinking between the polyrotaxane molecules led to hydrogel formation. Our group prepared a series of PEG hydrogels crosslinked by a hydrolysable polyrotaxane that has ester linkages at the both ends of PEG chain [26–28]. Ito and Okumura prepared polyrotaxane hydrogels that are crosslinked by cyanuric chloride [29]. In our case, the α-CDs in the hydrolysable polyrotaxane were linked with another PEG chains to form the crosslinked network. The major characteristic is that the erosion time is prolonged by increasing PEG/α-CD feed ratio. We observed that the hydrogels prepared the PEG/α-CD feed ratio below 2 were completely eroded. However, when the hydrogels were prepared so as to give the PEG/α-CD feed ratio above 2, the hydrogels were not eroded even in strong alkaline conditions [26, 28]. From this result, we hypothesized that tubular α-CD structure will be remained in the hydrogels after dethreading the PEG chain in the polyrotaxane.

In this study, the hydrogels were prepared by crosslinking α-CDs in the hydrolyzable polyrotaxane with PEG to array the α-CD, even after the ester linkages in the polyrotaxane were hydrolyzed in 0.1N NaOH. Tubular structure in the hydrogels was evaluated by incorporation behavior of sodium dodecyl sulfate (SDS) into the hydrogels, as compared with randomly crosslinked α-CD hydrogels. Furthermore, unsaturated fatty acids (palmitoleic acid and oleic acid) were applied to the hydrogels to compare difference of the alkyl chain length in the incorporation behavior.

2. Methods

2.1. Materials

The hydrolyzable polyrotaxane (Mₙ of PEG: 3300, the number of threading α-CD: 23) was prepared by our previous method [15, 28]. Amino-terminated PEG (PEG-bisamine, Mₙ = 600) was purchased from Suntechno Chemical Co. (Tokyo, Japan). Sodium dodecyl sulfate (SDS) and N,N-carbonyldiimidazole (CDI) was purchased from Wako Pure Chemical Co., Ltd (Osaka, Japan). Palmitoleic acid (PA) and Oleic acid (OA) were purchased from Sigma-Aldrich (St Louis, USA). Acetonitrile was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Dimethylsulfoxide (DMSO) was purchased from Wako Pure Chemical Co., Ltd (Osaka, Japan), and distilled by the usual method. All other chemicals used were of reagent grade.

2.2. Preparation of hydrogels

The PEG hydrogels crosslinked by the hydrolyzable polyrotaxane were prepared according to our previous method [28]. Briefly, a CDI-activated polyrotaxane (0.4 g) was dissolved in 10 ml of dry DMSO, and PEG-bisamine (1.04 g) was added to the solution (the PEG/α-CD feed ratio: 5). The mixture was degassed and injected in a Teflon spacer with two glass plates, the size of which was 20 cm × 20 cm × 0.08 mm. Then, melted PEG-bisamine was added to the mixture and kept at 40 °C for 24 h. The obtained hydrogels were washed with DMSO for 1 day and cut to 300 mm × 400 mm. The slab hydrogels were washed with water for 1 day and immersed in 0.1 M NaOH aqueous solution for 1 h at room temperature to hydrolyze the terminal ester linkage in the polyrotaxane to obtain a tubular α-CD-PEG hydrogel (MT-PEG hydrogel, Fig. 1(a)). In the similar manner, a PEG hydrogel crosslinked by α-CD (α-CD-PEG hydrogel) was prepared (CDI-activated α-CD: 0.34 g, PEG-bisamine: 1.04 g) (Fig. 1(b)). In this condition, α-CD content in feed was the same (the PEG/α-CD feed ratio: 5). The thicknesses of the tubular α-CD-PEG hydrogel and α-CD-PEG hydrogel were measured to be 89.8 ± 13.8 and 76.5 ± 5.7 µm.

2.3. Partition experiments

The partition experiment was carried out according to a report from Andreopoulos et al. with some modification.

![Fig. 1. Illustration of (a) tubular α-cyclodextrin-PEG hydrogel and (b) α-cyclodextrin-PEG hydrogel.](image-url)
SDS aqueous solution (0.7 mmol/l) or each fatty acid suspension (4.0 mmol/l) was prepared and vigorously stirred for 1 day at room temperature. The swollen hydrogels in a net were placed in the solution or suspension and the mixture was stirred under N₂ in the dark. Periodically, samples from the suspension were collected and applied to HPLC analysis. HPLC system equipped with an intelligent HPLC pump PU-980 and a 2-line degasser DG-980-51 was used for characterization (Japan Spectroscopic Co., Ltd). The signals were detected by a refractive index detector RI-930 (Japan Spectroscopic Co., Ltd). Each sample (40 µl) was diluted with acetonitrile (160 µl). After passing through a milipore filter, the solution is injected to a HPLC column (ODSpak·F411, Showdex, Tokyo, Japan) to determine the concentration of each fatty acid. The mobile phase was the mixture of acetonitrile and water (80:20) and the flow rate was 0.7 ml/min. Each sampling was triplicate. The concentration of the SDS and fatty acids was calculated by the peak integration with the calibration curve for each samples.

The partition coefficient (K) of the SDS and fatty acids was determined by the results of HPLC analysis using the following equations

\[ K = \frac{V_s C_m}{V_m C_e} \]

where \( V_s \) is the volume of the fatty acid solution (ml); \( C_i \), the initial concentration of the fatty acids (mg/ml); \( V_m \), the volume of the hydrogel (ml); \( C_e \) is the concentration of the surrounding SDS solution or the fatty acid suspension after equilibration.

3. Results and discussion

3.1. Effect of the tubular structure in the hydrogel on SDS partition

Fig. 2 shows incorporation profile of SDS into the MT-PEG and α-CD-PEG hydrogels at room temperature. The MT-PEG hydrogel incorporated SDS much faster than the CD-PEG hydrogel. The partition coefficient (K) of SDS to the MT-PEG and CD-PEG hydrogel was calculated to be 212 ± 27 and 143 ± 18, respectively. These results suggest the tubular structure, made from a template of the polyrotaxane, is much more attractive to include SDS in the α-CD cavities. In our previous study, a MT consisting of intermolecularly crosslinked α-CD molecules was purified by gel permeation chromatography, and inclusion complexation between the MT and SDS in water was analyzed by isothermal titration calorimetry to qualify the effect of the tubular structure of α-CDs on the incorporation of alkyl chains into the α-CDs cavity [24]. The association constant (Kₐ) of the MT was larger than that of α-CD. The magnitudes of the enthalpy change (\( \Delta H \)) and the entropy change (\( \Delta S \)) were found to be much larger than the α-CD/SDS system. Thus, the result of Fig. 2 was well consistent with the thermodynamic property of the MT/SDS system. The rapid incorporation of SDS into the MT-PEG hydrogel is considered to be due to the tubular structure in the MT-PEG hydrogel.

3.2. Effect of the alkyl chain length of unsaturated fatty acids on the partition

In order to demonstrate the alkyl chain separation system, we carried out the same experiments as the SDS/MT-PEG hydrogel system using fatty acids. Two kinds of unsaturated fatty acids (palmitoleic acid (C16:1) and oleic acid (C18:1))
and oleic acid (C18:1) were selected because such unsaturated fatty acids are expected as a functional food for human health and nutrition. Fig. 3 shows a marked decrease in the C16:1 and C18:1 concentration in the suspensions. The concentration of both fatty acids in the suspensions was decreased during 2 days and reached to the equilibrium concentration for 3–5 days. The K values of C16:1 and C18:1 were 552 ± 834 and 3841 ± 861, respectively. The K values of these fatty acids were one order larger in magnitude than the SDS/MT-PEG hydrogel system (Table 1). In the case of those fatty acids systems, absorption onto the surface of the MT-PEG hydrogel was observed. These results suggest that both the absorption and incorporation into the MT in the hydrogels are likely to contribute to the K values. However, the opaque medium of C16:1 changed to transparent after 4 days later. Such the transparency was not observed in C18:1/MG-PEG hydrogel system (data not shown). These observations are likely to correlate with the K values. Our previous results showed that the MT recognizes preferably longer alkyl chains, presumably due to enhanced hydrophobic interactions between the alkyl chains and the deeply hydrophobic cavity of α-CDs in the MT [25]. Comparing with the previous case, the results of Table 1, i.e. shorter chain length (C16:1) were more preferable than longer chain length (C18:1), were inconsistent with the MT property. Presumably, the tubular structure was broaden in relation to the surface absorption of the fatty acids, which may reduce the hydrophobic interactions in the α-CD cavities as seen in the MT with wider molecular weight distribution [24].

4. Conclusion

A new hydrogel having tubular structure of arrayed α-CDs crosslinked with PEG (MT-PEG hydrogel) was prepared using a hydrolyzable polyrotaxanes. The SDS incorporation into the MT-PEG hydrogel was faster than the α-CD-PEG hydrogel. The rapid incorporation of SDS into the MT-PEG hydrogel was strongly dependent to the tubular structure in the MT-PEG hydrogel. This incorporation property was also observed when unsaturated fatty acids were used in the system. Therefore, the tubular structure in hydrogels is advantageous for separating alkyl chains from aqueous solutions.

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