Preparation of Hydrolyzable Polyrotaxane Containing Ester Linkages and Its Degradation Behavior

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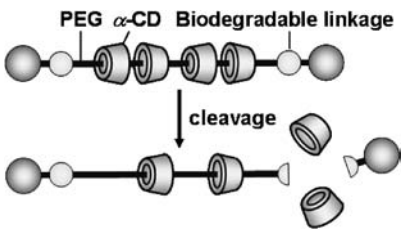
A hydrolyzable polyrotaxane composed of an ester-containing poly(ethylene glycol) chain and α-cyclodextrins showed gradual degradation into its water-soluble components in aqueous conditions, based on the dissociation of the polyrotaxane triggered by the hydrolysis of the ester groups.

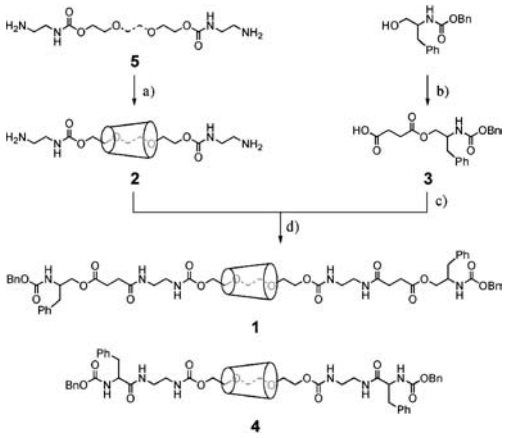
In the last several decades, biodegradable polymers have been studied as implantable materials for cell growth and tissue engineering.[1] These materials must satisfy various requirements such as sufficient mechanical strength, non-toxicity, and bioinertness before and after degradation for clinical use in a living body. In order to design and construct biodegradable materials,[2] some attention should be paid to the fact that the material has to have the potential to degrade perfectly in appropriate conditions, and that undesirable decomposition has to be avoided during the preparation and purification. It is of course necessary to purify the materials aimed at a clinical use in a living body. In this context, our approach for the design of biodegradable materials based on the structural features of polyrotaxanes, and especially on their dissociation, is promising. Stimuli-responsive biodegradable polyrotaxanes as shown in Scheme 1 would provide quite a new model as a mode of biodegradation.[3] Degradation based on the dissociation of polyrotaxanes, that is, the transformation of the supramolecular materials with a high molecular weight into water-soluble and bioinert components would be favorable in terms of effective degradation, low toxicity, and biocompatibility in a living body.

A polyrotaxane composed of a PEG chain containing ester groups at both ends and α-cyclodextrins (α-CDs) was designed and prepared as a candidate for biodegradable polymers, in which the ester group(s) would be expected to hydrolyze to trigger the following dissociation in response to pH. Thus, a successful preparation of the ester-containing polyrotaxane in spite of the potential of degradation[4] and its hydrolysis behavior were demonstrated.

The hydrolyzable polyrotaxane **1** was prepared by capping a pseudopolyrotaxane **2** with an ester-containing bulky *N*-benzyloxycarbonyl (Z-) phenylalanine-based succinic acid derivative **3**



Scheme 1. Biodegradation based on dissociation of polyrotaxane triggered by stimuli-responsive cleavage of biodegradable linkages.



Scheme 2. Preparation of hydrolyzable polyrotaxane **1** and chemical structure of ester-free polyrotaxane **4**: Reagents and conditions; a) α-CD, water; b) succinic anhydride, DMAP, pyridine (74%); c) *N*-hydroxysuccinimide, DCC, THF; d) DMF (15%).

in DMF as shown in Scheme 2. The pseudopolyrotaxane **2** was obtained by mixing a PEG chain **5** attached to amino groups at both ends with α-CD in water, followed by lyophilization according to a method reported by Harada et al.[5] The bulky capping molecule **3** was derived from a commercially available Z-phenylalaninol by treatment with succinic anhydride in pyridine containing a catalytic dimethylaminopyridine, and then employed as a succinimidyl succinate just before the capping reaction by condensation with **2**. Reprecipitation and dialysis using DMSO and water allowed **1** to be isolated without decomposition, which was confirmed by ^{1}H NMR and GPC measurements. The ester-free polyrotaxane **4** was also prepared as a reference by capping the pseudopolyrotaxane **2** with Z-phenylalanine in a similar manner to that used for **1**.

According to Figure 1a, notable broadening signals were observed for CD protons in **1**, which is characteristic of CD-based polyrotaxanes in DMSO-d_6.[5] Aromatic protons assigned to capping moieties in **1** were also detected. Figure 1b shows sharp signals as is observed for common pseudopolyrotaxanes without any terminal bulky groups implying the dissociation of **2** in DMSO-d_6 into the respective components α-CD and PEG. These observations clearly indicate that the polyrotaxane **1** was successfully prepared and isolated in pure form.[6] This was also confirmed by GPC measurements for **1**, **2**, and **4** eluted with DMSO, in which a shorter retention time (40 min) for the polyrotaxanes **1** and **4** was observed than for the α-CD (50 min) accompanied with the dissociation of pseudopolyrotaxane **2** in DMSO (Figure S1).[9]

The hydrolysis property of the ester-containing polyrotaxane **1** was investigated by monitoring the time change in the

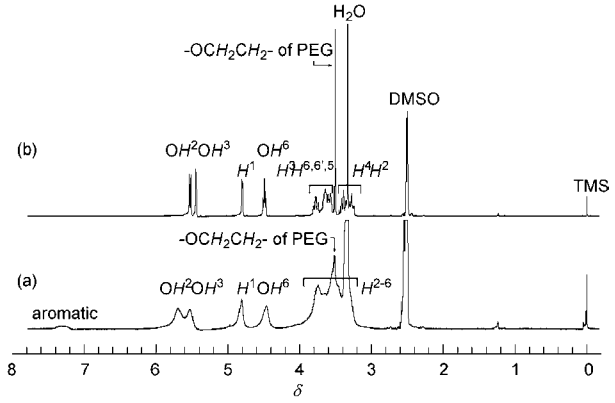


Figure 1. ^{1}H NMR spectra (300 MHz) of (a) polyrotaxane **1** and (b) pseudopolyrotaxane **2** in DMSO-d_6 at room temperature.

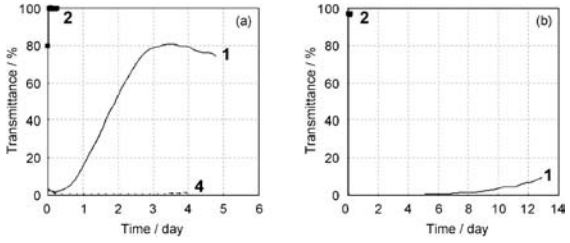


Figure 2. Continuous changes in transmittance of suspensions for **1** (thin line), **2** (bold line), and **4** (dashed line) at (a) pH 9.18 and **1** (thin line), and **2** (bold line) at (b) pH 6.86 at room temperature.

transmittance by comparison with that for the pseudopolyrotaxane **2** and the ester-free polyrotaxane **4**. It is difficult to estimate exactly the amount of cleaved ester linkages from the change in transmittance because the cleavage of both ester linkages is not required for triggering the dissociation. Each sample was suspended in a tetraborate pH standard solution adjusted to a pH of 9.18. A gradual increase in the transmittance at 500 nm from the baseline for **1** indicates qualitatively[7] that the water-insoluble **1** was gradually reduced by hydrolysis to produce water-soluble component(s), whereas an instantaneous change from a turbid suspension to a clear solution for **2** showed the dissociation of the inclusion complex. Any change in the transmittance for the ester-free polyrotaxane **4** was not found during the observation (Figure 2a).

In order to obtain further information on water-soluble component(s) in each suspension, several clear upper portions were collected at an arbitrary time for GPC measurements. The signals detected by RI for each portion collected from the suspension of **1** and **2** had the same retention time, indicating that at least one of the water-soluble components is α-CD (Figure S2).[9] The concentration of the PEG component in the portion was too low to be detected by RI on GPC measurements.[8] On the other hand, the portions collected from the suspension of **4** did not contain any water-soluble components, which means that it was intact under the conditions in accordance with the result of the transmittance measurements. These observations demonstrate that the ester-containing polyrotaxane **1** was hydrolyzed to produce its water-soluble components.

Hydrolysis under almost neutral conditions (pH 6.86) was also examined through transmittance measurement (Figure 2b). It then took much more time for the ester-containing polyrotaxane **1** to initiate hydrolysis than under basic conditions (pH 9.18), to reach only 10% even after 13 days.

In conclusion, we demonstrated the design and preparation of the hydrolyzable polyrotaxane **1** composed of an ester-containing PEG chain and α-CDs. The hydrolysis of **1** into its water-soluble components under aqueous conditions adjusted to both a basic and almost neutral pH was confirmed by monitoring continuous changes in transmittance and by GPC measurements of clear upper portions collected from suspensions. We are now studying the design of hydrogels based on hydrolyzable polyrotaxanes. It is easily conceivable that the dissociation of polyrotaxanes in hydrogel form into its components upon degradation of labile linkage(s) such as the ester group will be suitable for biocompatible materials such as scaffolds for tissue engineering.

References and Notes

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2 Aliphatic polyesters such as poly(lactic acid), and poly(glycolic acid) have been widely investigated to develop biomaterials which undergo hydrolysis for degradation. Recent reviews: a) Y. Tokiwa, A. Jarerat, *Biotechnol. Lett.* **2004**, *26*, 771. b) H. Tsuji, *Macromol. Biosci.* **2005**, *5*, 569. c) Y. Tokiwa, B. P. Calabia, *Appl. Microbiol. Biotechnol.* **2006**, *72*, 244. d) D. Chitkara, A. Shikanov, N. Kumar, A. J. Domb, *Macromol. Biosci.* **2006**, *6*, 977.

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4 A labile bond such as ester linkage was introduced prospectively into a capping agent not a CD-based pseudopolyrotaxane[5] which is commonly prepared in water. Another ester-containing polyrotaxane **1**′[9] had been designed in previous reports such as the following a) and b), however, the preparation and isolation of desired polyrotaxane **1**′ could not be achieved sufficiently due to the lack of attention to the potential of degradation. a) J. Watanabe, T. Ooya, N. Yui, *Chem. Lett.* **1998**, 1031. b) J. Watanabe, T. Ooya, N. Yui, *J. Biomater. Sci. Polym. Ed.* **1999**, *10*, 1275.

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6 The number of threading α-CD molecules in the obtained polyrotaxane **1** was calculated from the ratio of peak integrations for both C(1) protons in CD and methylene protons in PEG in the NMR spectrum measured in D_2O containing approximately 1 wt % NaOD to be ca. 18 (Figure S3a).[9]

7 A quantitative understanding for the dissociation of polyrotaxane is difficult because some inclusion complexes can be soluble in water when the number of threading α-CD molecules is small.

8 A PEG component in the clear upper portion was detectable by TLC on silica gel significantly. Also, after evaporation of the portion, the remaining solid was suspended in CH_2Cl_2. It was confirmed by ^{1}H NMR that the CH_2Cl_2 layer contained the PEG component.

9 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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