Preparation and Characterization of a Polyrotaxane with Non-enzymatically Hydrolyzable Stoppers

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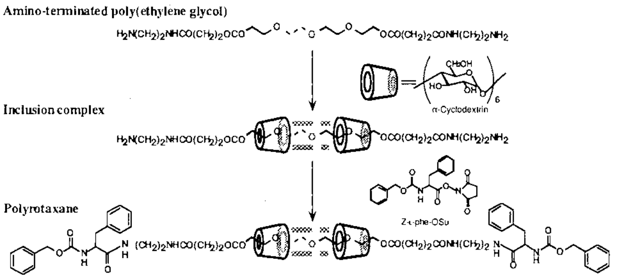
A degradable polyrotaxane, in which many α-cyclodextrins are threaded onto the poly (ethylene glycol) capped with L-phenylalanine via ester linkages, was synthesized and characterized for use as new implantable materials.

In the last decade, biodegradable polymers have been studied as implantable materials for cell growth and tissue regeneration.[1-3] Aliphatic polyesters such as poly(L-lactic acid) (PLA) is an example of implantable materials for tissue engineering.[4-6] By controlling the crystallinity and non-enzymatic hydrolysis, excellent mechanical properties of PLA have been demonstrated and maintained *in vivo* for several months. However, the high crystallinity of PLA reduces water intrusion into the crystalline regions resulting in incomplete hydrolysis; crystalline oligomers remain in the tissue for a long time. Such a complex situation causes chronic inflammatory reactions at the implanted sites.[7] Taking these problems into account, it is considered that ideal implantable materials should have the following two characteristics: one is excellent mechanical properties until the tissue is regenerated, and the other is rapid and perfect hydrolysis and absorption *in vivo* after the tissue regeneration.

In our recent studies, a novel design for a biodegradable polymer has been proposed by constructing a supramolecular structure of a polyrotaxane; many α-cyclodextrins (α-CDs) threaded onto a poly(ethylene glycol) (PEG) chain are capped with benzyloxycarbonyl (Z-) L-phenylalanine (L-Phe) via ester linkages. The field of supramolecular chemistry such as rotaxanes and polyrotaxanes has been developed from the viewpoint of the construction of new molecular architectures.[8-10] We have studied a biodegradable polyrotaxane consisting α-CDs, PEG and L-Phe.[11-13] From these studies, we established the design of biodegradable polyrotaxanes for biomedical applications such as drug carriers[11] and materials for blood contacting devices.[12,13] Our next concern of the biodegradable polyrotaxanes is the design of implantable materials for tissue engineering. The most favorable characteristics of the polyrotaxanes as implantable materials will involve their excellent biocompatibility, mechanical properties and instantaneous dissociation properties. The polyrotaxane may have high crystallinity which is due to intermolecular hydrogen bonds between the hydroxyl groups of the α-CDs. This high crystallinity is postulated to disappear due to terminal hydrolysis, although the crystallinity of the aliphatic polyesters usually increases as a result of their hydrolysis. The hydrolysis of the ester linkage in a certain PEG terminal will trigger rapid dissociation of the supramolecular structure into α-CDs, PEG and Z-L-Phe, thus, this may not induce any inflammatory reactions. We now report the synthesis of a degradable polyrotaxane and its supramolecular dissociation by terminal hydrolysis. In order to achieve the dissociation of the supramolecular structure via non-enzymatic hydrolysis, terminal degradable moieties were introduced via ester linkages. This system can be advantageous in the design of implantable materials for tissue engineering.



Scheme 1. Synthesis of a degradable polyrotaxane.

In order to introduce ester linkages at both terminals of PEG ($\overline{Mn}$ = 3300), terminal hydroxyl groups were carboxylated by using succinic anhydride. The terminal carboxyl groups of PEG were activated by *N*-hydroxysuccinimide using dicyclohexylcarbodiimide. An amino-terminated PEG was then synthesized using the activated PEG and ethylenediamine. Figure 1 shows the IR spectra of (a) PEG, (b) carboxy-terminated PEG and (c) amino-terminated PEG. It was observed that the IR spectrum of the carboxy-terminated PEG has a carbonyl band with a C-O stretching vibration at 1735 cm^{-1}. Moreover, the spectrum of the amino-terminated PEG showed absorption bands at 1641 cm^{-1} and 1545 cm^{-1} which are assigned to the C-O stretching band of amide I and the N-H bending vibration band of amide II, respectively. From these results, we confirmed the introduction of ester and amide bonds at the PEG terminals.

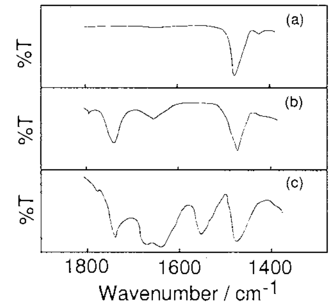


Figure 1. FT-IR spectra of poly(ethylene glycol) (a), carboxy-terminated PEG (b) and amino-terminated PEG (c).

An inclusion complex was prepared from α-CDs and the amino-terminated PEG, according to the method previously reported by Harada *et al.*[8-10] The end-capping reaction of the inclusion complex was carried out using the Z-L-Phe succinimide ester in DMSO according to our previous method.[11] Z-L-Phe was used as a terminal end-group because of preventing α-CD dethreading. From the ^{1}H-NMR spectrum of the polyrotaxane, peaks attributed to the α-CDs, PEG and aromatics of Z-L-Phe were confirmed.[14] The average number of α-CDs in the polyrotaxane was determined to be *ca.* 18-20 from the ^{1}H-NMR by comparing the integrations of the signals at 4.79 ppm (C(1)H of α-CD) with those at 3.51 ppm (CH_2 of PEG). The number of α-CDs was half the calculated number (*ca.* 39-40), assuming one α-CD molecule is threaded onto two repeating units of ethylene glycol.[9]

The crystalline structure of the polyrotaxane was confirmed using differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and wide angle X-ray diffraction (WAXD). From DSC and TGA curves, the polyrotaxane showed only one endothermic peak at 298 °C, indicating the decomposition of the polyrotaxane at this temperature. This result was very consistent with the results reported by Topchiva *et al.*[15] The WAXD pattern of the polyrotaxane revealed one main crystalline peak and two small crystalline peaks which is called a columnar structure (Figure 2c), and the pattern is different from α-CD and PEG (Figure 2a, 2b). These results suggest that polyrotaxane has a high crystallinity, which was very consistent with the results reported by Harada *et al.*[16]

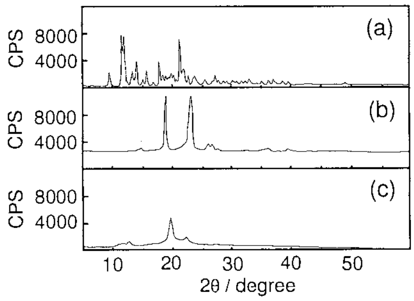


Figure 2. Wide angle X-ray diffraction patterns for α-CD (a), poly(ethylene glycol) (b) and the polyrotaxane (c).

It is important to demonstrate the supramolecular dissociation of the polyrotaxane by terminal hydrolysis, because the dissociation is expected to be applied to complete absorption *in vivo* at the implanted site. *In vitro* hydrolysis of the polyrotaxane was performed as follows: 30 mg of the polyrotaxane was dispersed in 3 ml of 0.1 M phosphate buffer (pH 7.4) and incubated at 37 °C. The α-CD release via supramolecular dissociation in the buffer was determined by phenol-H_2SO_4 assay.[17] Since the supernatant of the sampling solution was used in this assay, overestimation of the α-CD release due to α-CD in an inclusion complex and/or the polyrotaxane was confirmed to be negligible. In order to determine the hydrolysis of the ester linkage, carboxyl groups of the hydrolyzed Z-L-Phe derivative were allowed to react with 9-anthryldiazomethane (ADAM), and then, the ADAM-labeled Z-L-Phe derivative was determined by HPLC using a reverse phase column.[18]

As for the supramolecular dissociation of the inclusion complex (without the Z-L-Phe moiety), it was found that α-CD release was completed within 10 min. in the buffer at any pH (Figure 3a). On the other hand, as for the polyrotaxane, the completion of the α-CD release required several days (Figure 3b). These results indicate that the supramolecular dissociation of the polyrotaxane was prolonged in comparison with the inclusion complex. Further, the α-CD release rate at pH 8.0 was found to be faster than those at pH 7.4 and 5.7 (Figure 3b). The terminal hydrolysis at each pH was well correlated with the α-CD release behavior (data not shown). Taking these results into account, it is suggested that the terminal hydrolysis of the polyrotaxane is a dominant process in the supramolecular dissociation. Presumably, water intrusion into the terminal ester linkages may be related to the solubility of the polyrotaxane, which increases with ionization of the hydroxyl groups of the α-CD.

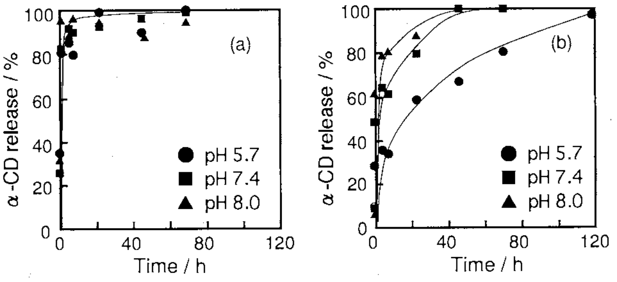


Figure 3. Cumulative α-CD release (%) from an inclusion complex (a) and the polyrotaxane (b).

In conclusion, we synthesized a degradable polyrotaxane with ester linkages. The polyrotaxane has a high crystallinity, and the supramolecular structure was completely dissociated by terminal hydrolysis. The control of water intrusion into the polyrotaxane will be a key parameter which determines the period of maintaining the supramolecular structure. Chemical modification of the polyrotaxane and its molding process are now in progress in order to control the intrusion of water into the ester linkages.

References and Notes

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14 ^{1}H-NMR (DMSO-d_6, 300 MHz) 8.00-7.80 (m, 3H×2, CONH of polyrotaxane), 7.35-7.20 (m, 10H×2, aromatic protons of Z-L-Phe), 5.50 (d, 6H×20, O(2)H of α-CD), 5.43 (m, 6H×20, O(3)H of α-CD), 4.79 (d, 6H×20, C(1)H of α-CD), 4.48 (t, 6H×20, O(6)H of α-CD), 4.10 (broad m, 4H×2, CH_2 of succinic acid moiety), 3.80-3.25 (m, 30H×20, C(3)H, C(5)H, C(6)H, C(4)H, and C(2)H of α-CD), 3.51 (s, 4H×75, CH_2 of PEG), 3.05 (m, 2H×2, CH_2 of L-Phe), 2.84 (s, 4H×2, CH_2 of ethylenediamine moiety)
^{13}C-NMR (DMSO-d_6, 75 MHz) 178.6 (COONH of Z group), 174.9 (CONH of L-Phe), 172.4 (ester group), 171.4 (CONH of ethylenediamine moiety), 143.9-126.7 (aromatic of Z-L-Phe), 102.3 (C(1) of α-CD), 82.4 (C(4) of α-CD), 73.6 (C(3) of α-CD), 72.5 (C(2), C(5) of α-CD), 71.6 (CH_2 of Z group), 70.1 (PEG), 60.4 (C(6) of α-CD), 53.4 (CH of L-Phe), 37.0 (CH_2 of L-Phe), 31.0 (CH_2 of ethylenediamine moiety), 29.0 (CH_2 of succinic acid moiety)
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17 α-CD in supernatant was hydrolyzed to be glucose by H_2SO_4. After the solution was mixed with 5 wt% phenol aqueous solution, glucose content in the solution was determined by the measurements of visible absorbance at 486 nm.
18 The measurement was carried out the following conditions: eluent: acetonitrile:H_2O = 8:2, flow rate: 0.6 ml/min., detection: fluorescence detector excited at 365 nm, emitted at 412 nm.