Block-Selective Movement of α-Cyclodextrins in Polyrotaxanes of PEI-*b*-PEG-*b*-PEI Copolymer

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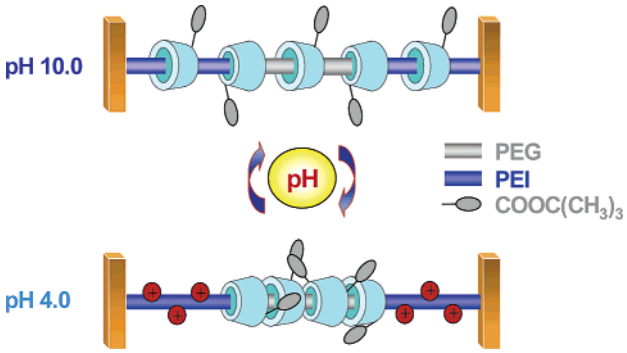
Many current supramolecular assemblies have been developed in nanoscale architectures, which can be precisely controlled in response to external signals such as pH, temperature, or the electric field.[1−14] Specifically, mechanically interlocked molecules such as catenanes and rotaxanes have received a great deal of attention in the design of molecular machines because of their ability to switch between two or more states.[1−3] Stoddart et al. reported on controlling the location of macrocycles between nonequivalent sites in catenane and rotaxane architectures.[4] Since the components of the rotaxanes are linked together by dynamic mechanical locks, the mobility of rotaxane can be controlled at the molecular level by applying external stimuli.[5,6] However, the molecular shuttles have been demonstrated in organic solvents, so that the biological applications in mild conditions are limited.[7,8]

To design functional supramolecular assemblies, which can be precisely controlled in response to external signals in mild conditions, various polyrotaxanes have been prepared using cyclodextrins (CDs) and water-soluble polymers.[9−11] In the polyrotaxane structure, CDs can translate around the threading guest polymer such as poly(ethylene glycol) (PEG), poly(propylene glycol) (PPG), or polyethylenimine (PEI) chains.[12,13] Along this line, recently, we reported a unique stimuli-responsive polypseudorotaxane between a PEI-*b*-PEG-*b*-PEI triblock copolymer and α-CDs, which showed block-selective inclusion complexation via pH variation.[14]

In this study, a pH-sensitive polyrotaxane was designed by end-capping the polypseudorotaxane of the PEI-*b*-PEG-*b*-PEI copolymer and α-CDs (Scheme 1). The solubility in aqueous solution was increased by eliminating the hydrogen bonding between the α-CDs by introducing *tert*-butoxy groups. As shown in Scheme 1, α-CDs in the water-soluble polyrotaxane showed pH-dependent movement along the PEI and PEG blocks, which was characterized by ^{1}H NMR and X-ray diffraction measurements.

Scheme 1. Schematic Illustration for the Mobility of Macrocycles in the Block-Selective Polyrotaxane via pH Variation



Experimental Part

Materials. Poly(ethylene glycol) (PEG, M_n 2 kg/mol) and α-cyclodextrin (α-CD) were purchased from Aldrich (Milwaukee, WI) and Wako Pure Chemical Co. (Tokyo, Japan), respectively, and they were purified by recrystallization in distilled water, followed by drying in vacuo at 100 °C. Mesyl chloride (MsCl), 2-methyl-2-oxazoline (OZ, distilled over calcium hydride), *N*,*N*′-carbonyldiimidazole (CDI), 1-(*tert*-butoxycarbonyl)imidazole, and ammonia solution (2.0 M in methanol) were purchased from Aldrich Co. Dimethyl sulfoxide (DMSO), triethylamine (TEA), acetonitrile, and methylene chloride were purchased from Wako and distilled over calcium hydride. Diethyl ether and other chemicals were used as received without further purification.

Synthesis of PEI-*b*-PEG-*b*-PEI Copolymer. The PEI-*b*-PEG-*b*-PEI copolymer was prepared according to our previous paper.[14] Briefly, PEG-bis(mesylate) (12.0 g, 0.005 mol) and OZ (20.4 g, 0.24 mol) were dissolved in dry acetonitrile (120 mL), and the temperature was increased up to 80 °C under a nitrogen atmosphere. After 24 h stirring, 15 mL of ammonia solution (0.03 mol) was added to the reaction mixture with stirring at room temperature for 6 h. The crude triblock copolymer, POZ-*b*-PEG-*b*-POZ, was then isolated by precipitation into cold diethyl ether (26.9 g, yield 83%). In the following step, the tertiary amide groups in POZ blocks were hydrolyzed by reflux in 10% HCl solution at 100 °C for 24 h. The reaction mixture was poured into cold acetone. The solid was collected by centrifugation, washed with distilled water, and then dried in vacuo at 40 °C to give the PEI-*b*-PEG-*b*-PEI copolymer (19.4 g, yield 71%, M_n 4 kg/mol, M_w/M_n = 1.21). ^{1}H NMR (POZ-*b*-PEG-*b*-POZ, D_2O): δ 3.54 (s, $-CH_2CH_2O-$), 3.38 (s, $-CH_2CH_2-$ of OZ), 1.94 (m, $-COCH_3$). ^{1}H NMR (PEI-*b*-PEG-*b*-PEI, D_2O containing 0.2 M DCl): δ 3.52 (s, $-CH_2CH_2O-$), 3.46 (s, $-CH_2CH_2NH-$).

Synthesis of Polyrotaxane. The polyrotaxane of PEI-*b*-PEG-*b*-PEI copolymer was prepared by mixing 12.0 g of α-CD and 1.08 g of copolymer in DMSO (2:1 stoichiometry between repeating units of PEI-*b*-PEG-*b*-PEI and α-CD), followed by capping with 10 equiv of Z-L-Tyr (0.84 g) at room temperature in the presence of 1.2 equiv of benzotriazol-1-yloxytris(dimethylamino)phosphomium hexafluorophosphate (BOP, 1.56 g), 1-hydroxybenzotriazole (HOBt, 0.43 g), and *N*,*N*′-diisopropylethylamine (DIEA, 0.41 g) in 150 mL of DMF. After stirring at room temperature over 12 h, the mixture was poured into excess methanol, filtered, and washed with acetone two times to remove BOP, HOBt, DIEA, and unreacted triblock copolymer. The resulting precipitate was dried in vacuo at 60 °C to obtain a tyrosine-terminated polyrotaxane (PRx-Tyr, 4.1 g, yield 78%, M_n 19 kg/mol, M_w/M_n = 1.43). The number and threading % of α-CD in the copolymer, calculated from the ^{1}H NMR spectra, were about 15.6 and 34.6%. ^{1}H NMR (DMSO-d_6): δ 7.45−6.45 (m, aromatics of Z-L-Tyr), 4.87 (s, H-1 of α-CD), 3.84−3.36 (m, H-3, 6, 5, 2, and 4 of α-CD), 3.54 (s, $-OCH_2CH_2-$ of PEG), 2.92 (s, $-CH_2CH_2NH-$ of PEI).

Water-Soluble Polyrotaxane. To prepare a water-soluble polyrotaxane, α-CDs in the polyrotaxane were modified with the predetermined amounts of 1-(*tert*-butoxycarbonyl)imidazole in the presence of 1.2 equiv of CDI and KOH. The mixture was stirred in DMSO at 60 °C for 12 h and precipitated into excess acetone, followed by dialysis (MWCO 15 000) against

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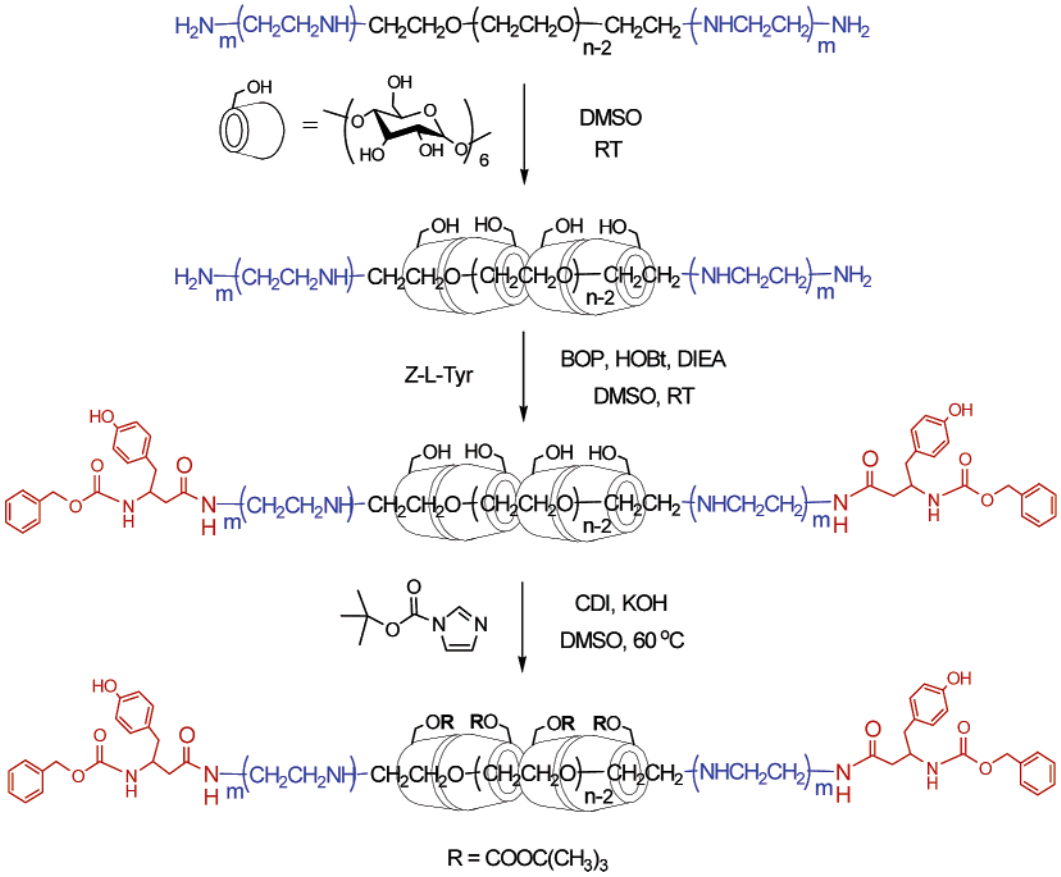

Figure 1. Synthetic routes for butoxy-PRx-Tyr composed of PEI-*b*-PEG-*b*-PEI copolymers and α-CDs.

water to give the *tert*-butoxy group conjugated polyrotaxane (butoxy-PRx-Tyr, 3.67 g, yield 81%, M_n 21 kg/mol, M_w/M_n = 1.45). The number-average molecular weight was estimated by ^{1}H NMR and GPC (eluent: 1% formic acid solution; standard: pullulan). The numbers of the butoxy groups per PRx-Tyr and α-CD were found to be about 7.8 and 0.49, respectively. ^{1}H NMR (D_2O at pH 10.0): δ 7.45−6.45 (m, aromatics of Z-L-Tyr), 4.88 (d, H-1 of CD), 3.85−3.31 (m, H-3, 6, 5, 2, and 4 of CD), 3.54 (s, $-OCH_2CH_2-$ of PEG), 2.54 (s, $-CH_2CH_2NH-$ of PEI), 1.43 (s, $-CH_3$ of *tert*-butoxy group).

Results and Discussion

To prepare a pH-sensitive polyrotaxane, a PEI-*b*-PEG-*b*-PEI copolymer was synthesized consisting of $EI_{22}EG_{45}$-EI_{22}. As shown in Figure 2a, the block compositions of PEG and PEI were tailored as the same length to give a full movement of α-CD rings by changing the environmental pH. The precipitated polypseudorotaxane was collected by ultracentrifuge, and both termini were capped with bulky Z-L-tyrosine groups, which decreased the water solubility significantly. To improve the water solubility of the prepared polyrotaxane, the α-CDs threaded along the PEI-*b*-PEG-*b*-PEI copolymer were modified with 1-(*tert*-butoxycarbonyl)imidazole. Since the *tert*-butoxy group is inert to the broad range of pH, it was expected that introducing small amount of butoxy groups to the α-CDs would increase water solubility of the polyrotaxane by cleaving the hydrogen bonds between α-CDs as well as not affecting the pH-sensitive mobility of α-CDs.[15] The chemical structure of the modified polyrotaxane was confirmed by ^{1}H NMR and GPC analyses. The degree of substitution (DS) of butoxy groups per α-CD and the molecular weight of butoxy-PRx-Tyr were calculated to be about 0.49 and 21 kg/mol, respectively.

^{1}H NMR spectra in Figure 2c,d show pH dependence of the resonance signals of the polyrotaxanes. At pH 10.0, all the signals of the polyrotaxane are clearly seen as in DMSO-d_6 (Figure 2b), indicating freely mobile state of α-CDs along the copolymer chains. It is interesting to note that, at pH 4.0, the resonance peaks ascribed to α-CD became broad, and each proton could not be pinpointed. This suggests the restricted molecular motion of α-CD molecules at a low pH, where the PEI block is ionized. ^{1}H NMR analysis provides some information on the localization or congestion of α-CDs somewhere in the polymer chains by adjusting pH below pH 4.0. According to our previous work, α-CDs were expelled from the ionized PEI chain due to the unfavorable interaction between two species.[14] Thus, this suggests high possibility of the selective movement of α-CDs onto the PEG middle block in the polyrotaxane.

The pH-dependent mobility of α-CDs was confirmed by spin−lattice (T_1) and spin−spin (T_2) relaxation times for C(1)H of α-CDs (δ = 4.88 ppm) using a 750 MHz NMR spectrometer at 25 °C. Relaxation times can provide significant information about mobility in dynamic ranges. As shown in Table 1, the α-CD movement in the butoxy-PRx-Tyr was compared with free α-CDs in terms of pH. Since the PRx-Tyr was not soluble clearly in water, the relaxation times could not be detected. At pH 10.0, the butoxy-PRx-Tyr showed relatively stable T_1 (1.882 s) and T_2 (0.076 s) values, which are similar to those of free α-CDs (1.826 and 0.081 s, respectively), reflecting the dispersion of α-CDs over the entire triblock chains. In contrast, at pH 4.0, the butoxy-PRx-Tyr exhibited a relatively high T_1 (2.411 s)

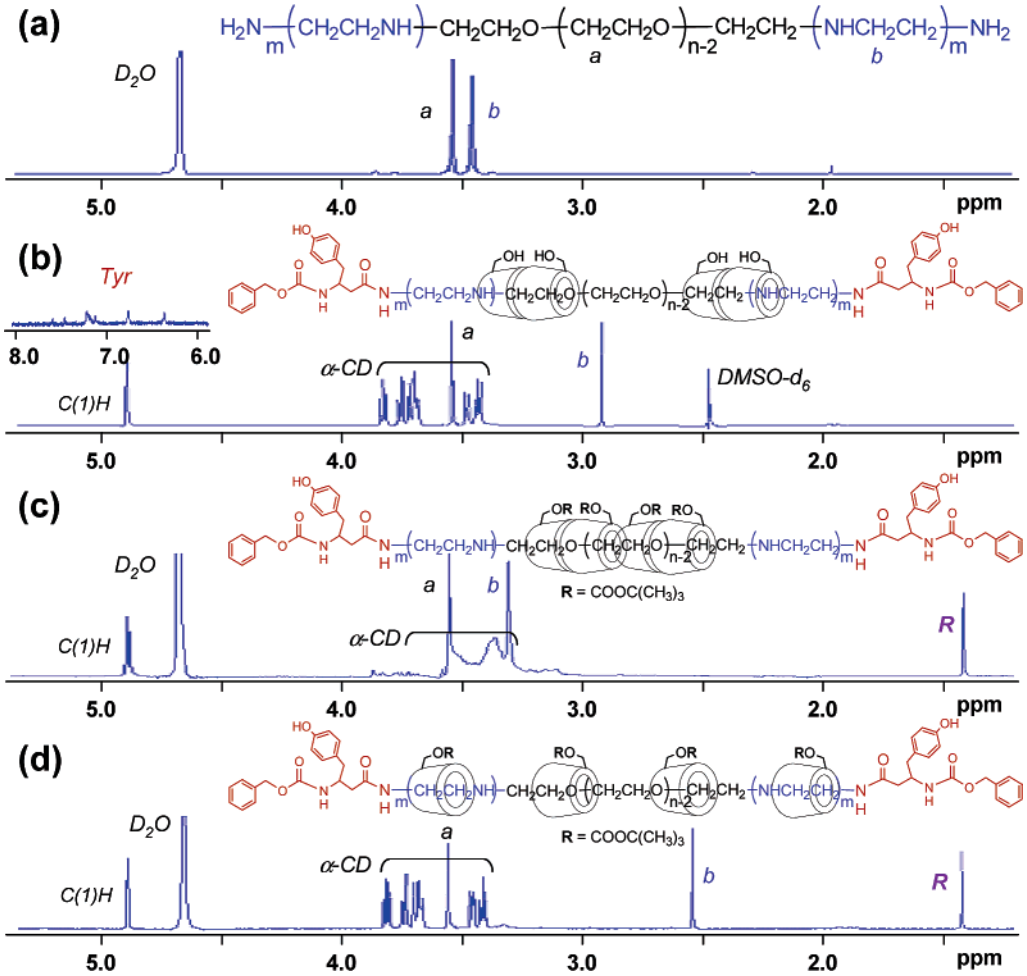

Figure 2. Partial ^{1}H NMR spectra of $EI_{22}EG_{45}EI_{22}$ copolymer in D_2O at pH 4.0 (a), PRx-Tyr in DMSO-d_6 (b), and butoxy-PRx-Tyr in D_2O at pH 4.0 (c) and at pH 10.0 (d). The solution pH was adjusted by adding DCl or NaOD in D_2O.

Table 1. Mobility of α-CDs in the Butoxy-PRx-Tyr by NMR Measurements in D_2O

samples	pH	T_1 (s)[a]	error (s)	T_2 (s)[a]	error (s)
α-CD	10.0	1.830	0.100	0.081	0.011
butoxy-PRx-Tyr[b]	4.0	2.410	0.110	0.049	0.006
	10.0	1.880	0.130	0.076	0.009

[a] The mobility of α-CD was estimated by the spin−lattice (T_1) and spin−spin (T_2) relaxation times for C(1)H of α-CDs (δ = 4.88 ppm) using a 750 MHz NMR spectrometer at 25 °C. [b] *tert*-Butoxy group conjugated polyrotaxane (yield 81%, M_n 21 kg/mol); the numbers of the butoxy groups per PRx-Tyr and α-CD, calculated from the ^{1}H NMR spectra, were about 7.8 and 0.49, respectively. The solution pH was adjusted by adding DCl or NaOD in D_2O.

and very low T_2 (0.049 s) values. These changes in relaxation times at low pH indicate that congestion of α-CDs in the middle PEG block, which may restrict the mobility of α-CD molecules.

The pH-dependent changes of the α-CD location were also confirmed by X-ray measurements. As shown in Figure 4b, the butoxy-PRx-Tyr at pH 4.0 exhibits a quite different pattern from either the multiple α-CD peaks (Figure 4e) or the diffuse halo of the triblock copolymer (Figure 4a). The two strong peaks at 2θ = 13.5° and 20.0° can be assigned as typical crystalline peaks of rotaxanated α-CDs (Figure 4d) via confinement of α-CDs to the middle PEG block, suggesting the electron density distribution of the core of α-CD molecules with radius ~5 Å.[16] On the other hand, some crystalline peaks at 2θ = 18.6°, 21.9°, and 22.7°, similar to the triblock copolymer, are observed in the butoxy-PRx-Tyr at pH

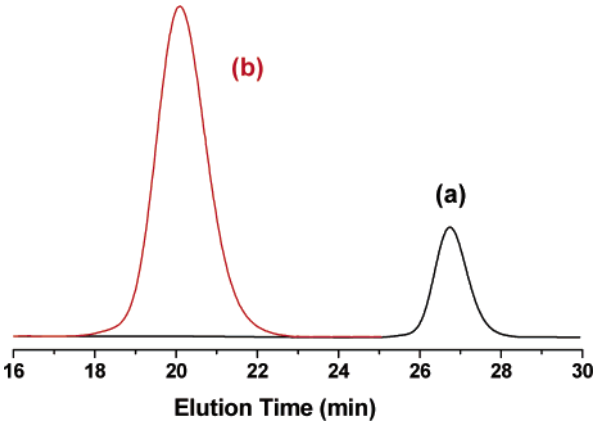

Figure 3. GPC traces of $EI_{22}EG_{45}EI_{22}$ copolymer (a) and butoxy-PRx-Tyr (b) in pure water containing 1% formic acid (standard: pullulan).

10.0 with a significantly decreased peak at 2θ = 20° (Figure 4c). It means that α-CDs are dispersed over the entire triblock copolymer, which significantly decreases the crystallization of α-CDs in the polyrotaxane structure. This result well supports the ^{1}H NMR data on the block-selective complexation of α-CDs with pH variation along the PEI-*b*-PEG-*b*-PEI copolymer chain.

In summary, we describe a new molecular shuttle system using the combination of α-CDs and the PEI-*b*-PEG-*b*-PEI copolymer. The appropriate design of stimuli-sensitive polymers and the control of interactions

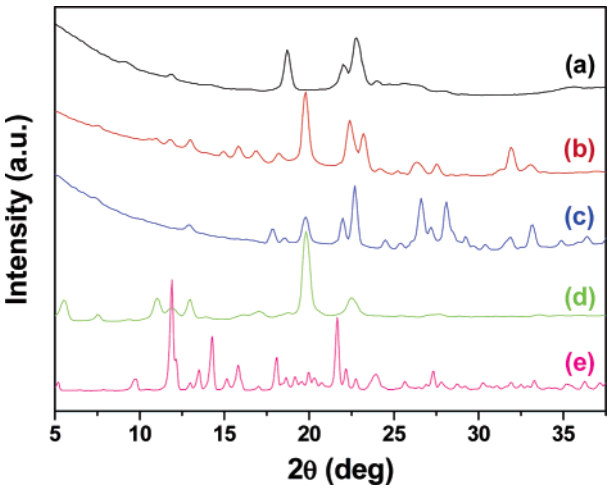

Figure 4. X-ray diffraction patterns of $EI_{22}EG_{45}EI_{22}$ copolymer (a), butoxy-PRx-Tyr at pH 4.0 (b), butoxy-PRx-Tyr at pH 10.0 (c), PEG/α-CD polyrotaxane (d), and α-CD (e).

between the interior of cyclic compounds and a linear polymer chain may extend the scope of this approach to a variety of novel supramolecular assembly systems.

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