Thermally Induced Localization of Cyclodextrins in a Polyrotaxane Consisting of β-Cyclodextrins and Poly(ethylene glycol)−Poly(propylene glycol) Triblock Copolymer

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ABSTRACT: A polyrotaxane, in which many β-cyclodextrins (β-CDs) are threaded onto a triblock copolymer of poly(ethylene glycol) (PEG) and poly(propylene glycol) (PPG) capped with fluorescein-4-isothiocyanate (FITC), was synthesized as a model of stimuli-responsive molecular assemblies for nanoscale devices. Coupling of FITC with the terminal amino groups in the polypseudorotaxane was performed in DMF at 5 °C. Under these conditions, a side reaction between the hydroxyl groups of β-CD and FITC was prevented. The interaction of the β-CDs with terminal FITC moieties in the polyrotaxane was significantly observed at low temperature. However, the interaction of the β-CDs with the PPG segment was observed with increasing temperature. On the basis of these results, it is concluded that the majority of the β-CDs move toward the PPG segment with increasing temperature although some β-CDs may reside on the PEG segments.

Introduction

New synthetic methods have been investigated over the past two decades in order to design "intelligent" or "smart" materials that exhibit large property changes in response to small physical or chemical stimuli. In particular, the progress in supramolecular chemistry has introduced a fascinating new field of macromolecular architecture mimicking supramolecular systems in nature. Inclusion complexes consisting of cyclic molecules and polymeric chains have been recently prepared as novel molecular assemblies.[1] The representatives are rotaxanes and polyrotaxanes, in which one and many cyclic molecules, respectively, are threaded onto a linear polymeric chain capped with bulky blocking groups. In the past decade, the field of rotaxanes and polyrotaxanes has been accelerated from the viewpoint of new molecular assemblies for nanoscale molecular devices. Stoddart et al. have developed molecular shuttles using rotaxanes in which a π-electron-rich macrocycle on a π-electron-deficient backbone moves reversibly back and forth in response to external stimuli.[2] Furthermore, other research groups have studied molecular shuttles. Nakashima et al. demonstrated a light-driven molecular shuttle utilizing α-cyclodextrin (α-CD) and azobenzene.[3] Mock et al. demonstrated a pH-driven molecular switch based on a ligand−receptor system utilizing a triamine ligand and alkanediamines.[4] Leigh et al. studied a peptide-based molecular shuttle in response to a change in the polarity of the environment.[5] Gibson et al., Ritter et al., and Harada et al. contributed to the novel development of polyrotaxanes. Recently, Gibson et al. studied solvent-switchable polyrotaxanes utilizing polyurethanes and a crown ether.[6,7] Ritter et al. studied side-chain polyrotaxanes utilizing CDs and a polymethacrylate or poly(ether sulfone) as the main chain.[8] Harada et al. investigated macromolecular recognition by CDs with poly(ethylene glycol) (PEG), poly(propylene glycol) (PPG), poly(isobutylene), and polyesters as a family of polyrotaxanes.[9]

We have studied biodegradable polyrotaxanes consisting of α-CDs and PEG for pharmaceutical and biomedical applications.[10] Our recent studies have focused on the design of stimuli-responsive polyrotaxanes consisting of β-CDs and a triblock copolymer as a candidate for biomimetic engineering materials.[11] Our concern regarding polyrotaxanes is how such a molecular assembly can be utilized as a material with molecular dynamic functions; threading many CDs onto a polyrotaxane might change the location along a linear polymeric chain in response to external stimuli which would be perceived as the action of mechanical pistons.

Because the driving force for such an inclusion complexation of CDs with a polymeric chain is due to intermolecular hydrogen bonding between neighboring CDs as well as steric fittings and a hydrophobic interaction between host and guest molecules,[9,12] several stimuli such as temperature and dielectric change may be used to control the assembled state of the CDs onto a polyrotaxane. We have found that a polyrotaxane consisting of many β-CDs and a triblock copolymer of PEG and PPG capped with naphthylamine exhibits the dynamic characteristics of a molecular piston; the soluble−insoluble nature of the polyrotaxane is controlled by temperature, presumably due to switching the assembled and dispersed states of the β-CDs along the triblock copolymer.[11] The changes in the soluble−insoluble nature are considered to be due to temperature-responsive changes in the location of the β-CD molecules along the triblock copolymer.

To achieve such a molecular piston function as a stimuli-responsive device, a polyrotaxane in which many β-CDs were threaded onto the triblock copolymer capped with fluorescein-4-isothiocyanate (FITC) was synthesized and characterized in terms of the change in the location of the β-CD molecules in response to temperature.[13] In our previous study, the polyrotaxane was synthesized by the addition reaction of the terminal

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amino groups of a polypseudorotaxane of β-CDs and the triblock copolymer with FITC in dimethylacetamide (DMAc) in the presence of triethylamine as a catalyst at room temperature. It was considered that side reaction between FITC and hydroxyl groups of a β-CD occurred in this synthetic condition. In this study, we succeeded in improving the synthesis of the polyrotaxane so as to minimize the side reaction. It was confirmed that the side reaction did not occur in the absence of the catalyst and at lower temperature. The polyrotaxane was soluble in polar solvents such as dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and 0.01 M NaOH over a wide range of temperatures, which means that the intermolecular forces between neighboring β-CDs were partially eliminated. Rather, a change in the hydrophobic interaction between the β-CD cavity and the PPG segment in the triblock copolymer dominates the location of the β-CDs in the polyrotaxane, which was analyzed by circular dichroism and 750 MHz ^{1}H NMR spectroscopies in weakly alkaline conditions.

Experimental Section

Materials. The PEG-*block*-PPG-*block*-PEG triblock copolymer (M_n = 4200, PPG segment M_n = 2250, PEG segment M_n = 975 × 2) was kindly supplied as a commercially available sample, named Pluronic P-84, from Asahi Denka Kogyo K.K., Tokyo, Japan. β-Cyclodextrin (β-CD), *N*,*N*′-carbonyldiimidazole (CDI), ethylenediamine, fluorescein-4-isothiocyanate (FITC), and sodium dodecyl sulfate (SDS) were purchased from Wako Pure Chemical Ind., Osaka, Japan. Tetrahydrofuran (THF) and dimethylformamide (DMF) were purchased from Wako Pure Chemical Ind., Osaka, Japan, and they were distilled by the usual methods. DMSO-d_6 and D_2O were purchased from Wako Pure Chemical Ind., Osaka, Japan, and NaOD (30 wt %) was purchased from Aldrich Chemical Co., Milwaukee, WI.

Measurements. Gel permeation chromatographic (GPC) analysis was carried out using a GF-710HQ column (Showa Denko Co., Ltd., Tokyo, Japan) with DMF + SDS (1 wt %) as the eluent. The eluent was detected with a refractive index detector (RI-930, Japan Spectroscopic Co., Ltd., Tokyo, Japan) and a UV−vis detector at a wavelength of 490 nm (UV-970, Japan Spectroscopic Co., Ltd., Tokyo, Japan). ^{1}H NMR spectra were recorded on 300 and 750 MHz FT-NMR spectrometers (Varian, Unity plus, CA) in DMSO-d_6 and D_2O + NaOD, respectively, at ambient temperature. The ^{13}C NMR spectrum was recorded on a 75 MHz FT-NMR spectrometer. 2D NOESY NMR experiments were carried out using a 750 MHz FT-NMR spectrometer at 25 °C. The 640 experiments were performed with 16 scans per experiment. Circular dichroism spectra were measured in 0.01 M NaOH and recorded on a circular dichrograph (J-720, Japan Spectroscopic Co., Ltd., Tokyo, Japan). For the measurement of the circular dichroism spectra, the temperature was regulated by a perche-type thermocontroller (EHC-441, Japan Spectroscopic Co., Ltd., Tokyo, Japan).

Preparation of Amino-Terminated Triblock Copolymer 3. Triblock copolymer (Pluronic P-84) **1** was dissolved in acetone and poured into an excess of cooled hexane. The purified **1** (4.1 g, 0.9 mmol) was dissolved in dry THF (10 mL) and added dropwise to excess *N*,*N*′-carbonyldiimidazole (CDI) (1.6 g, 9.9 mmol) in THF (25 mL) at room temperature for 6 h under a nitrogen atmosphere. After the solvent was evaporated from the reaction mixture, the resulting mixture was poured into excess diethyl ether to yield 3.9 g (91%) of CDI-activated triblock copolymer **2**. Then, **2** in dry THF (10 mL) was slowly added for 3 h dropwise into excess ethylenediamine (100 mol/mol of the triblock copolymer) at room temperature. Unreacted ethylenediamine was removed by evaporation, and the resulting viscous oil was dialyzed for 3 days against water. Finally, the dialyzed solution was freeze-dried to give **3** (2.2 g, 56%) as a slightly yellow liquid. IR (NaCl, cm^{-1}): 2862 (ν(C−H)), 1720 (ν(C═O)). ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 7.19 (t br, 2H, CONH), δ 3.40−3.50 (m, 4H × 44 and 3H × 39, −CH_2-CH_2O− of PEG and −CH_2CHO− of PPG), δ 2.96 (q, J = 5.7, 4H, CNCH_2), δ 2.53 (t, J = 5.9, 4H, CH_2N), δ 1.02 (d, J = 6.0, 3H × 39, PPG).

Preparation of Polypseudorotaxane 5. An aqueous solution of **3** (1.5 g, 0.3 mmol) was added to phosphate buffered saline solution (PBS: 35 mmol/L of KH_2PO_4, 65 mmol/L of Na_2HPO_4, pH 7.4, 380 mL) of β-CD **4** (6.8 g, 6.0 mmol) with gentle stirring at 40 °C for 48 h. The precipitate was isolated by centrifugation, washed with water, and dried in vacuo to yield 3.5 g (33%) of polypseudorotaxane **5**. The average number of β-CDs in **5** was ca. 17, which was calculated from the integral of the peak of β-CD (H-1) and that of the methyl protons on PPG segment in DMSO-d_6. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 5.71 (s br, 7H × 17, O-2 H of β-CD), δ 4.82 (d, J = 3.0, 7H × 17, O-1 H of β-CD), δ 4.45 (s br, 7H × 17, O-6 H of β-CD), δ 3.27−3.63 (m, 42H × 17, C-3 H, C-6 H, C-5 H, C-2 H, C-4 H of β-CD, 7H × 83, −CH_2CH_2O− of PEG and −CH_2-CHO− of PPG), δ 1.03 (d, J = 6.0, 3H × 39, CH_3 of PPG).

Synthesis of Polyrotaxane 7. Fluorescein-4-isothiocyanate (FITC) **6** (1.0 g, 2.3 mmol) was dissolved in DMF (3.0 mL). Then, **5** (3.0 g, 0.1 mmol) was added to this solution, followed by stirring at 5 °C for 72 h in the dark. The reaction mixture was poured into excess acetone to precipitate the product. The product was poured into excess water adjusted to pH 3−4 to yield the crude polyrotaxane. In this procedure, unreacted **6**, **4**, triethylamine, and FITC-introduced triblock copolymer **8** were confirmed to be dissolved in acetone, and furthermore, free β-CD was confirmed to be dissolved in the water adjusted to pH 3−4. Finally, the crude polyrotaxane was purified by gel permeation chromatography (GPC) on a Sephadex G-50 column (2.5 × 45 cm, Amersham Pharmacia Biotech, Tokyo, Japan) using DMSO as the solvent. The detection of the polyrotaxane was performed by absorbance at 405 nm. The yield was calculated from the feed of **3** to be 8%. The average number of β-CDs in polyrotaxane **7** was ca. 7, which was calculated from the integral of the peak of β-CD (H-1) and that of the methyl protons on PPG.

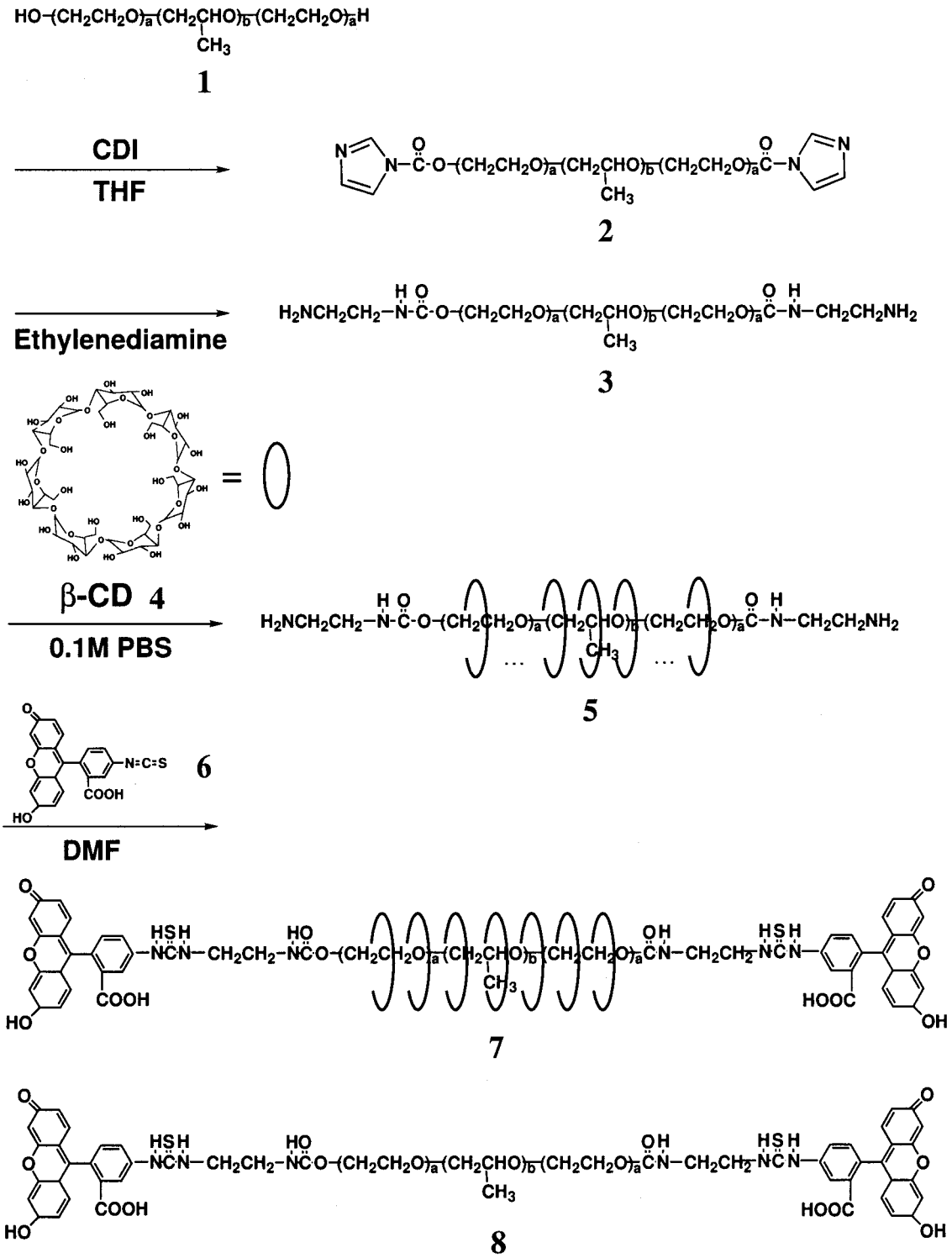
^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 6.66 (m, 6H, aromatic-H), δ 5.70 (m, 7H × 7, O-2 H of β-CD), δ 5.60 (m, 7H × 7, O-3 H of β-CD), δ 4.83 (d, J = 3.0, 7H × 7, O-1 H of β-CD), δ 4.45 (s, 7H × 7, O-6 H of β-CD), δ 3.17−3.69 (m, 42H × 7, C-3 H, C-6 H, C-5 H, C-2 H, C-4 H of β-CD, 7H × 83, −CH_2-CH_2O− of PEG and −CH_2CHO− of PPG). δ 1.03 (d, J = 6.0, 3H × 39, CH_3 of PPG). ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 101.99 (C-1 of β-CD), δ 81.71 (C-4 of β-CD), δ 74.96 (methine C of PPG) δ 73.19 (C-2 of β-CD), δ 72.81 (C-3 of β-CD), δ 72.11 (C-5 of β-CD), δ 60.08 (C-6 of β-CD), δ 17.62 (methyl of PPG). Combustion analysis. Calcd for $C_{547}H_{936}N_6O_{339}S_2 + 10H_2O$: C, 49.53; H, 7.21; N, 0.63. Found: C, 48.92; H, 7.95; N, 0.60.

Synthesis of Model Triblock Copolymer 8. Amino-terminated triblock copolymer **3** (1.5 g, 0.34 mmol) and FITC **6** (0.8 g, 2.1 mmol) in DMF (10 mL) were stirred in a nitrogen atmosphere at room temperature in the dark overnight. After the solvent was evaporated, the mixture was subsequently dialyzed for a week against weakly alkaline aqueous solution (pH 10). The product was freeze-dried to give a yellow viscous liquid (1.0 g) and finally purified by GPC on the Sephadex G-50 column (2.5 × 45 cm) using DMSO as the solvent (Yield 0.6 g, 34%). The detection of the model triblock copolymer was performed by absorbance at 405 nm. IR (NaCl, cm^{-1}): 2871 (ν(C−H)), (1716 (ν(C═O)). ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 6.66 (m, 6H, aromatic-H), δ 3.40−3.50 (m, 4H × 44 and 3H × 39, −CH_2CH_2O− of PEG and −CH_2CHO− of PPG), δ 1.02 (d, J = 6.0, 3H × 39, PPG). ^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 74.96 (methine C of PPG), δ 17.62 (methyl of PPG). Combustion analysis. Calcd for $C_{253}H_{446}N_6O_{94}S_2 + 10H_2O$: C, 57.13; H, 8.77; N, 1.58. Found: C, 56.64; H, 9.85; N, 1.37.

Results and Discussion

Preparation of Polypseudorotaxane 5 and the Synthesis of Polyrotaxane 7. To introduce FITC at both terminals of the triblock copolymer **1** via a thiocarbonyl linkage, the terminal hydroxyl groups in **1** were aminated using the *N*,*N*′-carbonyldiimidazole (CDI)-

Scheme 1



activated triblock copolymer **2** and ethylenediamine (Scheme 1). Polypseudorotaxane **5** consisting of β-CDs **4** and amino-terminated triblock copolymer **3** was obtained as a white powder in phosphate buffered saline (PBS), pH 7.4 at 40 °C. The yield of **5** in PBS at 40 °C was 33%. A much lower yield was obtained in an aqueous solution, pH 5.0 at 40 °C (6.2%), or in PBS at 10 °C (9.3%). Kaifer et al. have reported that complex formation between CDs and a polymeric chain with charged terminal groups is influenced by the pH of the solution.[14] Furthermore, increased temperature will contribute to the hydrophobic interaction of β-CDs **4** with the PPG segment in amino-terminated triblock copolymer **3**. Our results suggest that an increase in the solution temperature and the use of the salt solution contribute to enhancing the complexation of β-CDs **4** with **3**.

Polyrotaxane **7** was synthesized in DMF at 5 °C. In our previous study, the synthesis of the polyrotaxane was carried out in the presence of triethylamine as a catalyst at room temperature.[13] In this study, triethylamine was not used because it catalyzes the reaction of the isothiocyanate group of FITC with not only the terminal amino groups of polypseudorotaxane **5** but also

Table 1. Model Reaction of β-CD and FITC[a]

run no.	β-CD (g)	molar ratio of β-CD:FITC	temp (°C)	solvent[b]	yield[c] (%)
1	0.5	1:1	25	DMF + TEA	2.9
2	0.5	1:1	5	DMF + TEA	4.5
3	0.5	1:1	25	DMF	1.5
4	0.5	1:1	5	DMF	0

[a] The model reaction was carried out for 24 h. The reaction mixture was poured into excess acetone, and further the resulting precipitate was poured into excess water adjusted to pH 3−4 to yield the product. The yields were measured by weight. [b] Two milliliters of DMF, triethylamine (TEA) as a catalyst. [c] Calculated as 100% yield of one β-CD molecule conjugated with one FITC molecule.

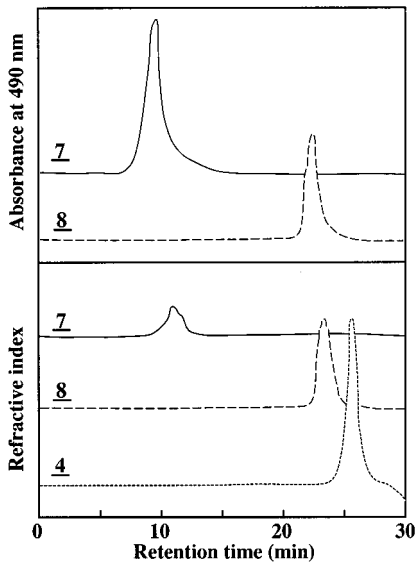


Figure 1. GPC traces of polyrotaxane **7**, model triblock copolymer **8**, and β-CD **4** which were detected by a UV−vis detector (490 nm) and refractive index detector in DMF + SDS at 30 °C.

the hydroxyl groups of β-CDs **4**. Furthermore, the lower temperature is likely to minimize the reaction between FITC **6** and β-CDs **4**. The influence of the triethylamine and the temperature on the reaction was confirmed by model reactions between the β-CDs and FITC in DMF (Table 1). The side reaction of β-CDs **4** and FITC **6** was prevented in the absence of triethylamine as a catalyst at 5 °C. These results indicate that the side reaction between FITC **6** and the hydroxyl groups of β-CD **4** was prevented.

The purity of polyrotaxane **7** was confirmed by GPC using DMF + sodium dodecyl sulfate (SDS, 1 wt %) as the eluent. SDS was added in order to prevent the association of polyrotaxanes **7** and model triblock copolymers **8** in DMF. As shown in Figure 1, the final product was detected by absorbance at 490 nm and the refractive index as a single peak on the GPC chart. A single peak at the same retention time in Figure 1 was also observed for polyrotaxane **7** in DMF without SDS (data not shown). Furthermore, the retention time of the final product was significantly shorter than that of β-CD **4** and model triblock copolymer **8**. From these results, it is obvious that there is no free β-CD in the final product. In the elemental analysis, the obtained data were consistent with the calculated value. From the ^{1}H NMR spectrum of **7** (Figure 2), all the peaks attributed to β-CDs, CH_2 of PEG and PPG, CH_3 of PPG, and aromatic protons of the terminal FITC were confirmed. Further, the introduction of FITC **6** only at both terminals of polypseudorotaxane **5** was also confirmed. The integral ratio of methyl protons of the PPG segment and aromatic protons of the terminal FITC moiety (methyl protons/aromatic protons = ca. 9.39) was consistent with the calculated value (ca. 9.75). The threading of β-CD was confirmed by chemical shifts of the ^{1}H NMR (Figure 3) and 2D NOESY NMR spectra (Figure 4). The peak of the inner-cavity H-3 protons of β-CD **4** in **7** was shifted 0.09 ppm to a higher field in comparison with the peak of free β-CDs; however, the protons located around the periphery of β-CD **4**, H-2 and H-4, were hardly shifted and broadened (Figure 3). Wenz et al. reported that threading of heptakis(2,6-di-*O*-methyl)-β-CDs onto poly(iminoundecamethylene) showed a shift in the H-3 protons.[15] Also, Stoddart et al. reported the shift of protons when β-CD formed a 1:1 complex with a guest molecule in D_2O.[16] From Figure 4, it was found that the signals of H-3 and H-5 protons of β-CD **4** were correlated with the resonance of methyl protons of the PPG segment. Therefore, the results of the H-3 proton shift and 2D NOESY NMR spectrum indicate the threading of β-CD **4** onto the triblock copolymer in **7**.

From the ^{1}H NMR spectrum, the average number of β-CDs in polypseudorotaxane **5** was determined to be ca. 17, almost the same as the stoichiometric number, ca. 20, assuming that one β-CD molecule is threaded onto two repeating units of propylene glycol.[9,17] On the other hand, the average number of β-CDs in polyrotaxane **7** was determined to be ca. 7. Intermolecular forces such as hydrogen bonding between neighboring β-CDs and/or hydrophobic interaction may be eliminated in DMF. Harada et al. reported that hydrogen bonding between neighboring β-CDs was a driving force for polypseudorotaxane formation with PPG.[9] Furthermore, the threading of CDs onto a linear polymer chain is believed to be kinetically controlled.[12,18] Taking these reports into account, the threading/dethreading process of β-CDs onto the triblock copolymer in DMF is likely to be kinetically controlled.

Polyrotaxane **7** was soluble in DMSO, DMF, and 0.01 M NaOH but not soluble in phosphate buffered solution (pH 7.4). This solubility of polyrotaxane **7** was different from that of the previously synthesized polyrotaxane.[13] Enhanced solubility of the previously synthesized polyrotaxane may be related to the excess introduction of FITC moieties into β-CDs.

Change in the Location of β-CDs in Response to Temperature. To clarify a change in the location of β-CDs **4** in polyrotaxane **7** in response to temperature, the interaction of β-CDs **4** with the terminal FITC moiety and with the PPG segment was analyzed by means of circular dichroism spectra in 0.01 M NaOH and the 750 MHz ^{1}H NMR spectra in D_2O + NaOD, respectively. The pK_a values of hydroxyl groups of β-CD and carboxyl group of FITC are ca. 12.2 and ca. 4−5 at 25 °C, respectively.[19] In 0.01 M NaOH aqueous solution (ca. pH 11.8), about 50% of hydroxyl groups of β-CD might be ionized. Thus, polyrotaxane **7** was soluble due to ionization of the carboxyl groups in FITC **6** and elimination of the intermolecular forces between neighboring β-CDs **4**. The higher field shift of H-3 protons in **7** was confirmed in D_2O + NaOD after 2 weeks at 25 °C. This result suggests that terminal thiocarbonyl linkage in **7** was stable in the weakly alkaline condition. As reference compounds, the circular dichroism and ^{1}H NMR spectra of model triblock copolymer **8** and β-CD were also measured under the same conditions.

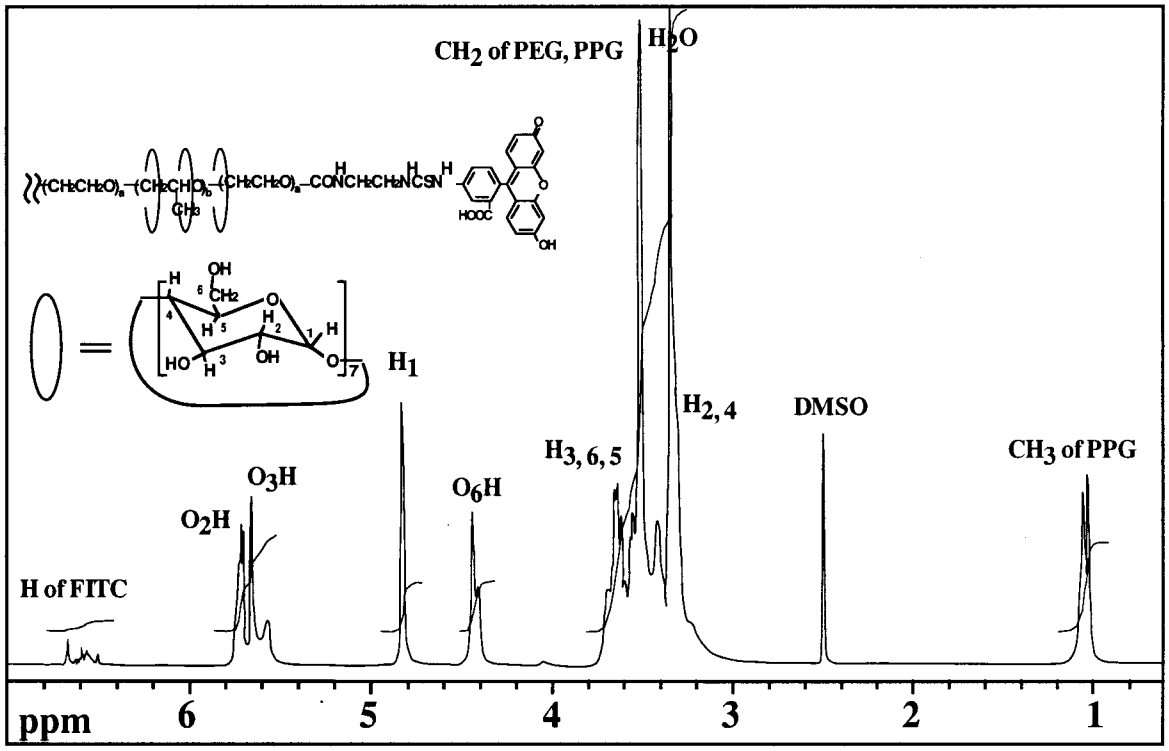


Figure 2. The 750 MHz ^{1}H NMR spectrum of polyrotaxane **7** in DMSO-d_6.

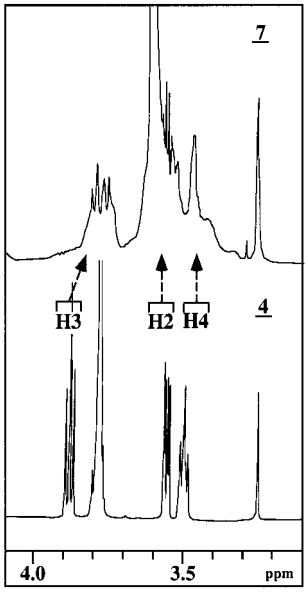


Figure 3. The 750 MHz ^{1}H NMR spectra of polyrotaxane **7** and free β-CD **4** in D_2O + NaOD.

The visible absorption and induced circular dichroism (ICD) spectra of polyrotaxane **7** are shown in Figure 5. The ICD spectra of **7** showed a positive ellipticity [θ] around at 490 nm, which corresponds to the visible absorption. As reference compounds, the circular dichroism spectra of model triblock copolymer **8** and β-CD **4** were also measured in 0.01 M NaOH. The ICD spectrum around the visible absorption at 490 nm was not observed in **8** in the presence of β-CD, and there was no ICD peak for β-CD (data not shown). From these results, it is suggested that the positive ellipticity [θ] around 490 nm of polyrotaxane **7** is due to the interaction of the terminal FITC moiety with β-CDs **4** threaded onto the triblock copolymer.

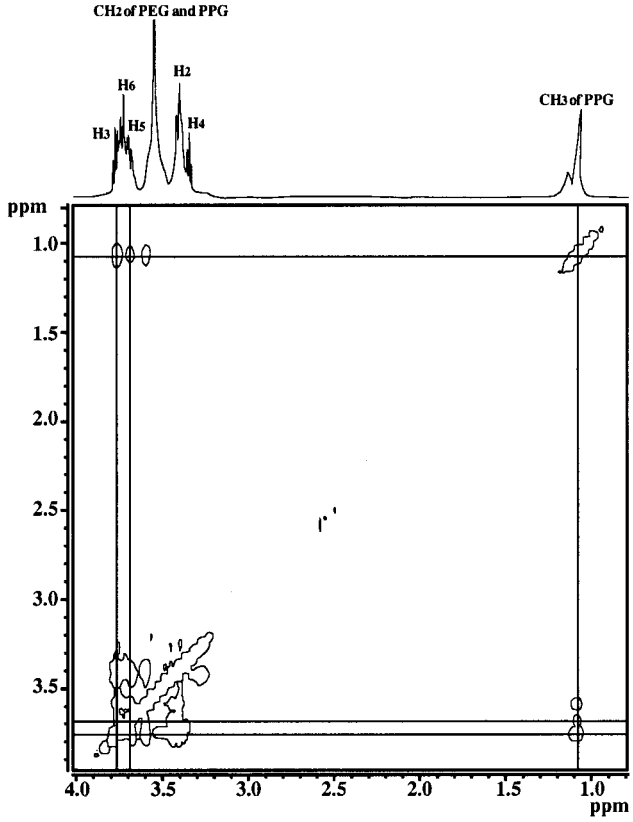


Figure 4. 2D NOESY NMR spectrum of polyrotaxane **7** in D_2O + NaOD.

The positive ellipticity [θ] of polyrotaxane **7** around 490 nm decreased when the temperature increased from 20 to 40 °C (Figure 5). A similar phenomenon in a temperature-dependent ICD intensity change has been reported for an inclusion complex between α-CD and *m*- or *p*-nitrophenol, indicating the dissociation of the complex with increasing temperature.[20] From this, the

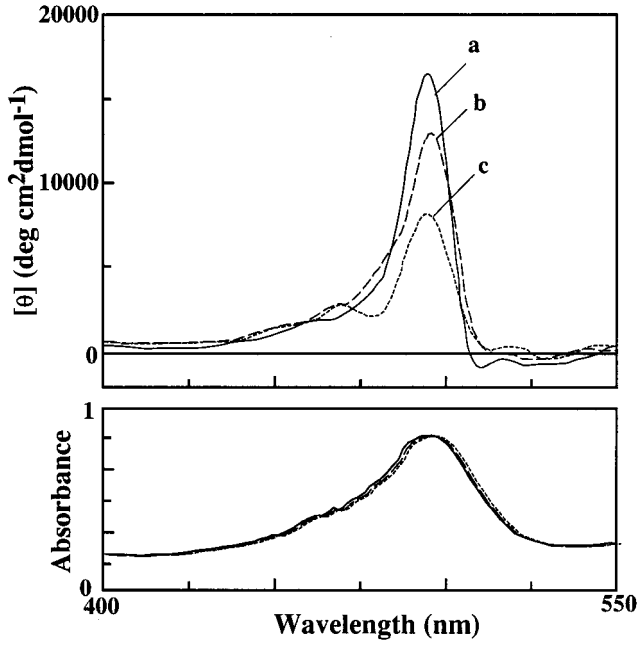


Figure 5. CD (top) and visible absorption (bottom) spectra of polyrotaxane **7** (2.5×10^{-5} M) at 20 (a), 30 (b), and 40 °C (c) in 0.01 M NaOH.

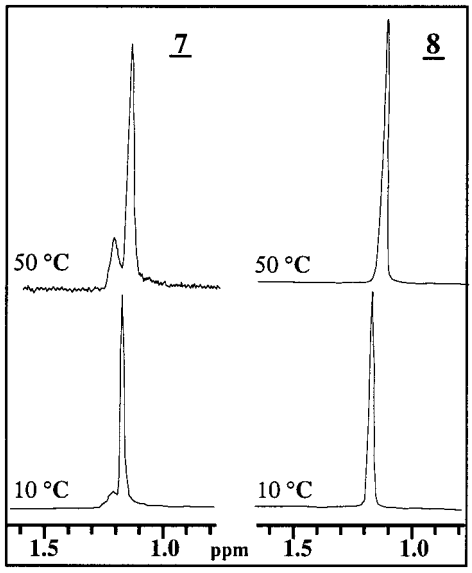


Figure 6. Temperature dependence (at 10 and 50 °C) of the 750 MHz ^{1}H NMR spectra of polyrotaxane **7** and model triblock copolymer **8** in D_2O + NaOD (0.01 wt %).

decrease in the ICD intensity suggests enhanced thermal motion of the β-CDs along the triblock copolymer. Observed ICD spectra around 470 nm at 40 °C may be related to the interaction between some β-CDs **4** and the terminal FITC moiety in **7**. Therefore, the interaction between β-CDs **4** and the terminal FITC moiety in **7** is considered to decrease with increasing temperature.

The interaction of the β-CD hydrophobic cavity with the PPG segment in polyrotaxane **7** was analyzed using 750 MHz ^{1}H NMR spectroscopy in D_2O + NaOD. With increasing temperature, the peak of the methyl protons in the PPG segment shifted and slightly broadened for polyrotaxane **7** (Figure 6). As for model triblock copolymer **8**, the peak of the methyl protons in the PPG segment was completely shifted to a higher field with increasing temperature; however, no broadening of the signal was observed (Figure 6). Harada et al. reported

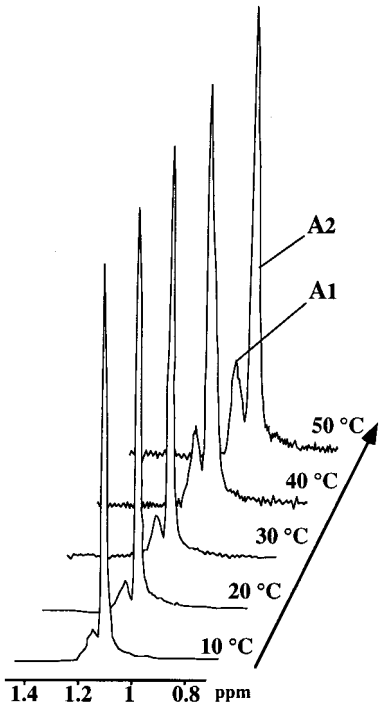


Figure 7. Temperature dependence of ^{1}H NMR peak shapes of the methyl protons in polyrotaxane **7**.

that the methyl and methylene protons of side alkyl groups in a polymer chain were shifted to a lower field by the addition of α-CDs in D_2O.[21] This result indicates that α-CDs include the side alkyl groups in the polymer chain. In addition, the peak broadening indicates that CDs are threaded onto the polymeric chains.[22,23] Harada et al. reported that the polyrotaxane consisting of many α-CDs and the PEG capped with dinitrophenyl groups shows the broadening of ^{1}H NMR signals because the movements of the molecules are restricted.[22] Taking these reports into account, the methyl proton peak shifted to the lower field and broadened for **7** is considered to be due to the interaction between propylene glycol (PG) units and the cavity of the β-CDs. To estimate the number of β-CDs **4** located on the PG units, the relative area of the shifted methyl proton peak (A_1) was calculated in the temperature range from 10 to 50 °C based on Figure 7. In this case, it is assumed that the shifted methyl proton peak is attributed to the included PG units with β-CDs **4**. The relative A_1 peak area was calculated by the following equation:

$$\text{relative } A_1 \text{ peak area (\%)} = 100 \times A_1/(A_1 + A_2) \quad (1)$$

where A_2 is the area of the original methyl proton peak. If all the threading β-CDs **4** (ca. 7) are localized on the PPG segment, the relative A_1 peak area can be maximally 35.9% assuming one β-CD molecule is threaded onto two PG units (total units of PG: ca. 39). The value of the relative A_1 peak area increased from ca. 6.9% to ca. 26.6% with increasing temperature from 10 to 50 °C (Table 2). At 50 °C, the relative A_1 peak area (26.6%) corresponds to 74% of the maximum peak area (35.9%). From this, it is considered that ca. 5.2 β-CD molecules were localized on the PPG segment at 50 °C. Presumably, the number of β-CD molecules on the PPG segment will be determined by the balance of the following two forces: the enhanced hydrophobic interaction between the β-CD cavity and the PPG segment and the repulsive

Table 2. Temperature Dependence of the Relative Peak Area and the Calculated Number of β-CDs

temp (°C)	rel peak area of A_1 (%)[a]	calcd no. of β-CDs on the PPG segment[b]
10	6.9	1.3
20	7.3	1.4
30	11.3	2.2
40	21.0	4.1
50	26.6	5.2

[a] Calculated by eq 1. [b] Calculated by the maximum peak area: 35.9%.

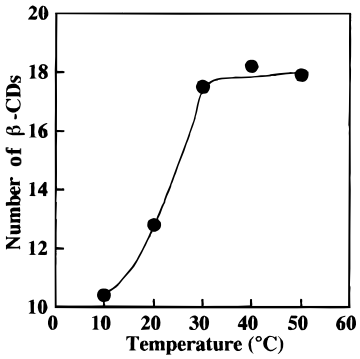


Figure 8. Effect of temperature on the formation of the polypseudorotaxane between β-CDs **4** and triblock copolymer **1** in 0.01 M NaOH for 48 h.

forces between the ionized hydroxyl groups in β-CD under the weakly alkaline condition. The movement of β-CDs toward the PPG segment with increasing temperature is supported by the enhanced polypseudorotaxane formation of β-CDs with triblock copolymer **1** at elevated temperature.

Figure 8 summarizes the temperature dependence of the polypseudorotaxane formation between β-CDs and triblock copolymer **1** in 0.01 M NaOH. A temperature rise was found to contribute to an increase in the number of threaded β-CDs in the polypseudorotaxane (molar ratio of the triblock copolymer/β-CDs in feed is 1/20). However, a stoichiometric number of threaded β-CDs (ca. 20) was not obtained even as the temperature increased to 50 °C. In a previous study, the effect of temperature on the polypseudorotaxane formation between triblock copolymer **1** and β-CDs was examined in 0.1 M PBS.[13] In that case, a linear relationship between the temperature and the number of threaded β-CDs was observed, and the polypseudorotaxane with a stoichiometric number of threaded β-CDs was obtained at 50 °C. Thus, the smaller number of threaded β-CDs at 50 °C in this study is considered to be due to the repulsive forces between the ionized hydroxyl groups in the β-CDs. Overall, it is considered that the hydrophobic interaction between β-CDs **4** and the PPG segment in **1** plays a dominant role in the complexation and is enhanced by temperature. Polypseudorotaxane formation was not observed above 60 °C due to the aggregation of **1** (the cloud point is ca. 60 °C in 1% solution). Therefore, it is concluded that the majority of β-CDs **4** move toward the PPG segment in **7** with increasing temperature, although some β-CDs **4** may reside on the PEG segments. Thus, it is demonstrated that the assembled and dispersed states of the β-CDs along the triblock copolymer are thermally switchable in polyrotaxane **7** (Figure 9).

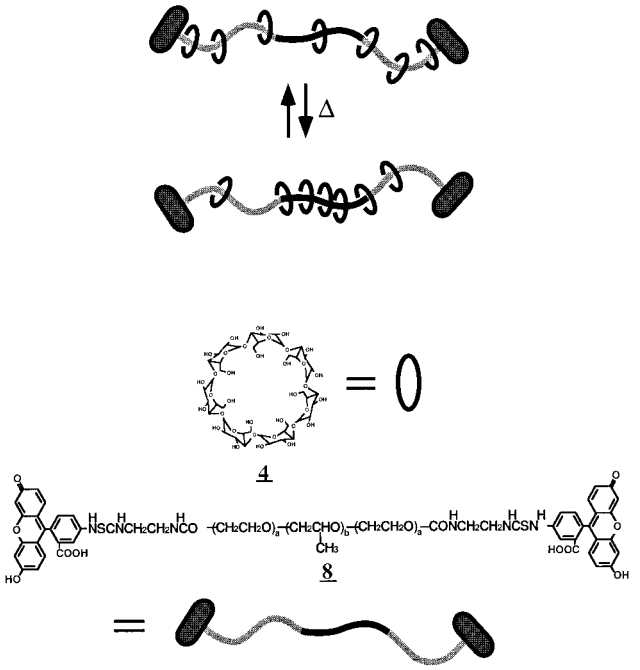


Figure 9. Thermoresponsive characteristic of polyrotaxane **7**.

Conclusion

Polyrotaxane **7** consisting of β-CDs **4** and a triblock copolymer **1** capped with FITC **6** was synthesized, and a change in the location of β-CDs **4** in response to temperature in 0.01 M NaOH was analyzed. Polyrotaxane **7**, in which ca. 7 β-CD molecules were threaded onto the triblock copolymer (M_n = 4200), was synthesized without side reactions. From the results of the induced circular dichroism and 750 MHz ^{1}H NMR spectroscopy, interaction of β-CDs **4** with the terminal FITC moiety in **7** was significantly observed at low temperature. However, with increasing temperature, a significant interaction of the β-CDs **4** with the PPG segment in **7** was observed. This phenomenon was supported by enhanced polypseudorotaxane formation at elevated temperature. Therefore, it is concluded that the majority of the β-CDs move toward the PPG segment with increasing temperature, although some β-CDs may reside on the PEG segments.

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