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One-pot synthesis of cyclodextrin-based radial poly[n]catenanes

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Supermolecules such as rotaxanes and catenanes have recently attracted considerable attention due to their potential widespread applications in areas such as molecular machines and switches. Moreover, the development of polyrotaxanes and polycatenanes, comprising multiple cyclic compounds, has allowed the fabrication of structures with novel properties. Although rotaxanes and polyrotaxanes have been extensively prepared from cyclodextrins as building blocks, very few studies have considered the syntheses of cyclodextrin-based polycatenanes. Here we report the one-pot syntheses and isolation of cyclodextrin-based radial polycatenanes with large numbers of cyclic components (>10) attached to a poly(ethylene glycol)–poly(propylene glycol)–poly(ethylene glycol) copolymer core, with characterization performed using Raman spectroscopy, gel permeation chromatography, ^{1}H-NMR spectroscopy, and other techniques. Overall, the results presented herein may be used to develop advanced supramolecular structures and materials, such as molecular machines, molecular actuators, molecular switches, biomaterials, and drug carriers.

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Mechanically interlocked molecules such as rotaxanes[1–5] and catenanes[6–8] have attracted considerable attention due to their unique properties and have been widely utilized for the assembly of molecular machines, switches, and motors[9–12]. In addition, polyrotaxanes[13–16] and polycatenanes[17,18], comprising multiple cyclic compounds, have been the subject of extensive research, since their polymeric structures exhibit novel properties. In particular, Harada's group has performed breakthrough research on polyrotaxanes[19]; these materials subsequently found numerous applications as topological gels[20,21], lithium ion batteries[22], and biomaterials[23–25]. However, few studies have considered the development of polycatenane-based materials because of the laborious syntheses involved.

Polycatenanes are classified as main-chain, side-chain, connected, radial, and network types (Supplementary Fig. 1)[17], and the syntheses of main-chain polycatenanes having a linear-chain type structure are the most challenging. Recently, however, Wu et al. reported the preparation of main-chain type polycatenanes that included 4–130 cyclic compounds[26]. Although the polycatenanes obtained were mixtures of linear, branched, and cyclic polycatenanes, this report provided access to main-chain-type polycatenanes and their preparation. In addition, a number of side-chain and network polycatenanes have also been reported[17,18]. By comparison, the syntheses and isolation of radial polycatenanes are challenging[17] due to the requirement of threading a linear polymer through several cyclic compounds (i.e., polypseudorotaxane formation) and the subsequent chain cyclization. Although high-dilution conditions favor cyclization, they concomitantly promote the dissociation of the polypseudorotaxane, which is a typical issue associated with the above synthesis method. To the best of our knowledge, the maximum number of components cyclized into a radial polycatenane reported thus far is seven (i.e., [7]catenane)[18,27–29].

Cyclodextrins (CyDs) (Supplementary Fig. 2) are cyclic compounds commonly used to fabricate interlocked molecules[9,13,30–32]. In 1981, Ogino first prepared a rotaxane-containing α-CyD and α, ω-diaminoalkanes capped with *cis*-$[CoCl_2(en)_2]Cl^2$, and Harada et al. successfully synthesized an α-CyD-containing polyrotaxane in 1992[19], with a large number of CyD-based polyrotaxanes reported thereafter[13,31,33]. In contrast to CyD-based rotaxanes and polyrotaxanes, the syntheses of the corresponding catenanes and polycatenanes are surprisingly difficult[34]. In 1958, Lüttringhaus et al. first attempted to prepare a CyD-based catenane by cyclizing an inclusion complex between a dithiol and α-CyD[35], which, unfortunately, failed. Thirty-five years later Stoddart's group successfully synthesized and isolated CyD-based [2] and [3]catenanes[36]. However, few studies on CyD-based catenanes have been reported since then[37–39]. In addition, the number of CyDs cyclized into catenanes is less than three (i.e., [2] or [3]catenanes)[36–39]. Notably, even fewer studies have reported CyD-based polycatenanes. Harada et al. reported the possible formation of a CyD-based polycatenane during the polymerization of 9-anthracene-capped α-CyD/poly(ethylene glycol) (PEG) polyrotaxane, which, to the best of our knowledge, is the only example of a CyD-based polycatenane[40]. However, these researchers did not separate the polycatenanes from the poly(polyrotaxane) because their similar properties precluded polycatenane isolation from the crude product. Therefore, the isolation and characterization of CyD-based polycatenanes have not been fully reported, necessitating further searching for facile synthesis and isolation methods to accelerate the development of polycatenane-based materials. Unlike polyrotaxanes, polycatenanes have no endcap molecules, which often limit water solubility, non-metabolic, expensive, and harmful. Thus, polycatenanes have the potential to function as safe, inexpensive, and green materials.

In view of this background, we here report the facile syntheses and isolation of CyD-based radial polycatenanes composed of more than 10 CyD rings. CyD-based polypseudorotaxanes (as opposed to polyrotaxanes) are strategically cyclized to yield polycatenanes, whereas non-cyclized polypseudorotaxanes are easily removed from the reaction system via dissociation in dimethyl sulfoxide (DMSO). For cyclization, the axial molecule (e.g., a PEG–poly(propylene glycol)–PEG copolymer (Pluronic P123, PEG–PPG–PEG)) is terminated with thiol groups, which enables disulfide bond formation under oxidizing conditions.

Results

Preparation of polycatenanes. We first tested α-CyD as the cyclic compound and PEG dithiol (HS–PEG–SH) as the axial compound because they readily form a water-insoluble polypseudorotaxane[41]. However, no polycatenane was obtained following oxidation with hydrogen peroxide (H_2O_2) (Table 1). Here, thiol group content in the reaction mixture was almost zero (data not shown), indicating the successful disulfide bonds formation. Therefore, no formation of α-CyD polycatenane with HS–PEG–SH was probably due to unsuccessful cyclization. In this case, α-CyD fully covered the PEG main chain and its terminals, thereby decreasing the mobilities of the PEG thiol groups (Supplementary Fig. 3a).

Hence, polycatenanes were prepared using β-CyD and Pluronic P123 (PEG–PPG–PEG; M_w of PEG ≈ 880 × 2 Da, M_w of PPG ≈ 4060 Da), as β-CyD forms a water-insoluble polypseudorotaxane with PPG[42] but not with PEG[13], to afford a polypseudorotaxane molecule with flexible terminal PEG chains (Supplementary Fig. 3b). PEG–PPG–PEG dithiol (HS–PEG–PPG–PEG–SH) was first prepared by activating the terminal hydroxyl groups of PEG–PPG–PEG with 1,1′-carbonyldiimidazole (CDI) followed by reaction with cystamine (Supplementary Fig. 4). The reaction product was subsequently reduced with 1,4-dithiothreitol (DTT). The resulting HS–PEG–PPG–PEG–SH was mixed with an aqueous solution of β-CyD to afford a polypseudorotaxane

Table 1 Yields and compositions of CyD-based polycatenanes prepared using HS-PEG-SH or HS-PEG-PPG-PEG-SH

Polymer	Terminal group	CyD	Average number of CyDs[a]	Coverage (%)[b]	Yield (%)	M_n (kDa)[c]
HS-PEG-SH	SH	α-CyD	-	-	0	-
HO-PEG-PPG-PEG-OH	OH	β-CyD	-	-	0	-
HS-PEG-PPG-PEG-SH	SH	α-CyD	-	-	0	-
		β-CyD	13.5	38.4	11.6	21.1
		γ-CyD	22.9	65.1	1.6	35.5

[a]Average number of CyD units per one axile molecule in the polycatenane
[b]Coverage = 2 × (CyD per PPG)/(PPG repeat units), assuming two PPG repeat units per CyD
[c]Number-average molecular weight calculated by ^{1}H-NMR analysis

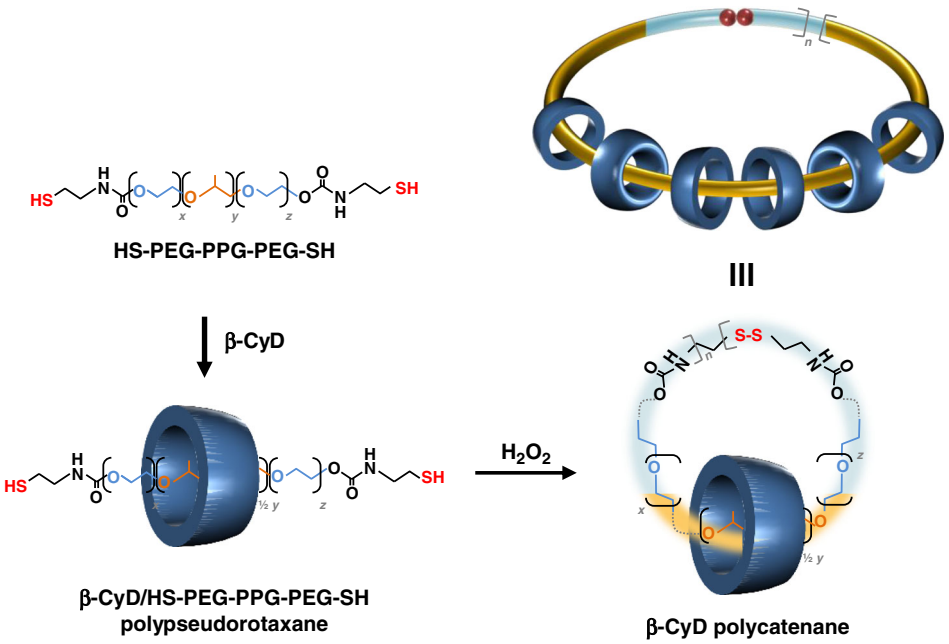

Fig. 1 Preparation of a β-CyD-based polycatenane. To remove the remaining polypseudorotaxane and free HS–PEG–PPG–PEG–SH and β-CyD, the crude product was dissolved in DMSO and washed with water and acetone

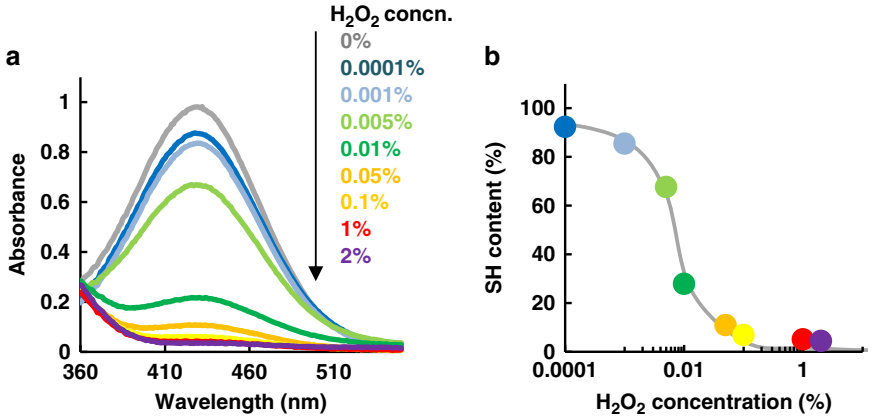

Fig. 2 Thiol-group content in the reaction suspensions. **a** Absorbance of 2-nitro-5-thiobenzoic acid and **b** thiol-group content in suspensions obtained by treating the prepared polycatenane with H_2O_2 solutions of varying concentration

precipitate after 24 h (Fig. 1). Subsequently, H_2O_2 was added to the suspension to oxidize the thiol groups of HS–PEG–PPG–PEG–SH and cyclize the polypseudorotaxane by disulfide bond formation.

To confirm the oxidation of the terminal thiol groups of the β-CyD polypseudorotaxane and following the addition of H_2O_2 solutions of varying concentration and the dissolution of the suspension in DMSO, the thiol group content of the reaction mixture was determined using Ellman's reagent (sulfhydryl-assay reagent, $\lambda_{max} = 325$ nm), which reacts with thiol groups to afford 2-nitro-5-thiobenzoic acid ($\lambda_{max} > 412$ nm). As shown in Fig. 2a, the addition of H_2O_2 decreased the absorbance of 2-nitro-5-thiobenzoic acid in a concentration-dependent manner, indicating that the thiol group content of the suspension decreases as the oxidation progresses. Furthermore, the addition of >1% v/v H_2O_2 reduced the thiol group content to almost zero (Fig. 2b), indicating that the thiol groups of HS–PEG–PPG–PEG–SH in the polypseudorotaxane formed disulfide bonds through cyclization and/or polymerization (Supplementary Fig. 5).

The unreacted polypseudorotaxane and/or polymerized linear polypseudorotaxane (i.e., poly(polypseudorotaxane)) were removed from the mixture by dissolving the obtained precipitate in DMSO to accelerate their dissociation (Supplementary Fig. 5). Subsequently, the above-mentioned DMSO solution was added dropwise to excess acetone, and the obtained precipitate was washed with water and acetone to remove unreacted and/or dissociated β-CyD and HS–PEG–PPG–PEG–SH, which was then lyophilized. Notably, new spot was observed at Rf = 0, and negligible amount of free β-CyD was present in the precipitate, as evidenced by thin-layer chromatography (TLC, Supplementary Fig. 6). These results suggest the successful synthesis of β-CyD polycatenane. Moreover, γ-CyD/HS–PEG–PPG–PEG–SH polypseudorotaxane also afforded a precipitate, indicating the formation of γ-CyD polycatenane. In contrast, no precipitate was obtained from the β-CyD/HO-PEG–PPG–PEG–OH and α-

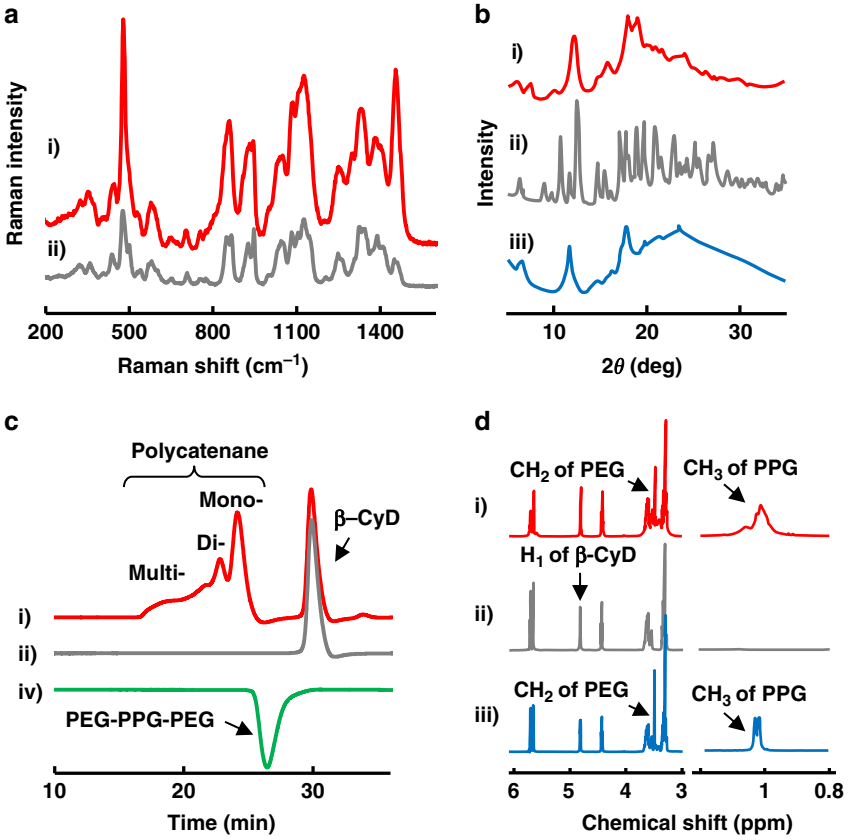

Fig. 3 Characterization of polycatenane. **a** Raman spectra, **b** powder X-ray diffraction patterns, **c** GPC profiles, and **d** ^{1}H-NMR spectra of: (i) reaction product (β-CyD-based polycatenane), (ii) β-CyD alone, (iii) β-CyD/PEG–PPG–PEG polypseudorotaxane, and (iv) PEG–PPG–PEG. The GPC traces were obtained using a refractive index detector

CyD/HS–PEG–PPG–PEG–SH polypseudorotaxanes after washing with DMSO, water, and acetone (Table 1).

Structural analysis of polycatenanes. To confirm successful synthesis of the polycatenane, the precipitate was first analyzed by Raman spectroscopy (Fig. 3a). Compared to that of β-CyD, the Raman spectrum of the precipitate exhibited broader peaks, probably owing to the formation of inclusion complexes between β-CyD and PEG–PPG–PEG[43].

CyD complexes exhibit three crystal structures: channel-type, layer-type, and cage-type. Polycatenanes as well as polypseudorotaxanes and polyrotaxanes are expected to exhibit channel-type structures[19,44]. Hence, powder X-ray diffraction was used to confirm the crystal structure of the precipitate (Fig. 3b). As expected, the aforementioned precipitate exhibited a diffraction pattern corresponding to a channel-type structure similar to that of β-CyD/PEG–PPG–PEG polypseudorotaxanes, which served as the positive control, whereas an entirely different pattern consistent with a cage-type structure was observed for β-CyD. Notably, the polypseudorotaxane and poly(polypseudorotaxane) in the crude product were removed by washing with DMSO, water, and acetone, which strongly suggests that the diffraction pattern of the precipitate corresponds to polycatenanes with channel-type structures.

In order to confirm the formation of polycatenanes, the precipitate was subjected to gel permeation chromatography (GPC) in DMSO (Fig. 3c). The results revealed the presence of broad peaks corresponding to polycatenanes composed of one mono-polycatenane, two di-polycatenanes, and a number of multi-polycatenanes which are formed from the cyclization of a single, two, or multiple polypseudorotaxanes, respectively (Supplementary Fig. 7), suggesting the successful size-based separation. The number-average molecular weight (M_n) of the mono-polycatenane was estimated to be 18.0 kDa, suggesting the presence of ~11 interlocked β-CyD molecules (Table 2). In addition, the mono-polycatenanes and di-polycatenanes showed low polydispersity index (M_w/M_n), suggesting the relatively homogeneous β-CyD units threaded into the polycatenanes. Importantly, a peak corresponding to free β-CyD was observed in the GPC trace of the polycatenane. Prior to GPC analysis, the sample solution was filtered through a 0.22 μm filter, which required the application of a large amount of force. Consequently, high-molecular-weight compounds were likely removed prior to GPC; these measurements therefore overestimate the actual free β-CyD content indicated by the TLC results (Supplementary

Table 2 Characterization of β-CyD-based polycatenanes by GPC

Polycatenane	Peak area (%)	M_n (kDa)[a]	M_w/M_n[b]
Mono-	30.6	18.0	1.05
Di-	16.1	37.1	1.03
Multi-	22.8	129.2	36.4

[a]Number-average molecular weight estimated by GPC
[b]Polydispersity index

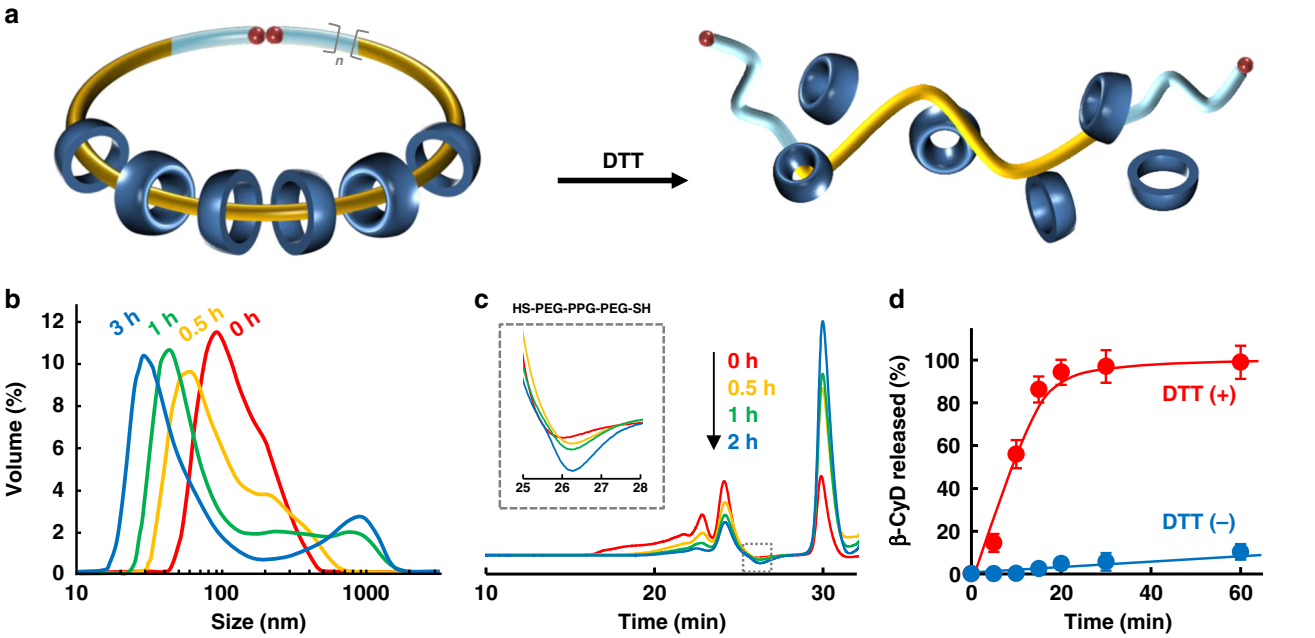

Fig. 4 Release of β-CyD from the polycatenane. **a** Schematic release of β-CyD from a polycatenane upon reduction. **b** Particle size distributions of β-CyD polycatenanes in the presence of DTT. **c** GPC profiles of β-CyD-based polycatenanes after treatment with DTT. **d** β-CyD-release profiles from polycatenanes in the absence/presence of DTT. Each data point corresponds to the mean ± standard error of three experiments

Fig. 6) and underestimate the high-molecular-weight-polycatenane content. Nevertheless, these results confirm the successful formation of polycatenanes. Currently, our group is working toward purifying the resultant products to obtain the mono-polycatenanes, and selective preparation of mono-polycatenanes is also ongoing.

Atomic force microscopy (AFM) was employed to examine the morphology of the reaction product and confirm its cyclic structure. The theoretical size of the prepared polycatenane is ~15 nm, which complicates direct AFM observation. Nevertheless, although AFM imaging indicated a size somewhat larger (~25 nm) than that predicted theoretically, the presence of cyclic compounds was confirmed (Supplementary Fig. 8). The above discrepancy is caused by the movement of the AFM cantilever and included its girth.

Subsequently, ^{1}H-NMR spectroscopy was employed to examine the polycatenane stoichiometry (Fig. 3d) and revealed the presence of peaks corresponding to both β-CyD and PEG–PPG–PEG. Notably, peaks corresponding to PPG were broadened due to its complexation with β-CyD. The average number of β-CyD units per polycatenane was determined to be 13.5, which corresponds to 38.4% coverage assuming two PPG repeat units per β-CyD. Based on the stoichiometry determined by ^{1}H-NMR integration, the molecular weight of the β-CyD-based polycatenane was calculated to be 21.1 kDa (Table 1), which is in good agreement with the GPC results. Moreover, the average number of γ-CyD units per polycatenane was also determined to be 22.9 with 65.1% coverage (Supplementary Fig. 9). Furthermore, the yields of the β-CyD-based and γ-CyD-based polycatenanes, containing 13.5 and 22.9 cyclic components on average, were estimated to be 11.6% and 1.6%, respectively (Table 1).

Release of β-CyD from polycatenane in the reduced state. Since the polycatenanes prepared herein contain disulfide bonds, we examined the release of β-CyD upon reduction (Fig. 4a). The sizes of the polycatenane particles were first determined in the presence of DTT (Fig. 4b); scattering data revealed the presence of aggregates with diameters of ~90 nm that decreased in size in a time-dependent manner following the addition of DTT. Moreover, the intensities of the GPC peaks corresponding to the polycatenanes decreased, whereas those of the peaks corresponding to β-CyD and HS–PEG–PPG–PEG–SH increased in the presence of DTT (Fig. 4c) and tris(2-carboxyethyl)phosphine hydrochloride (TCEP), an alternative reducing reagent (Supplementary Fig. 10). Moreover, after DTT treatment, TLC revealed a spot corresponding to β-CyD (Supplementary Fig. 6). The amount of β-CyD released from the polycatenane precipitate was subsequently determined by polarimetry (Fig. 4d). Although 10% of β-CyD was released at 60 min in the absence of DTT, probably due to the instability of disulfide bonds in water, the above-mentioned release was markedly accelerated by the addition of DTT. Hence, these results indicate that the prepared polycatenanes exhibit interlocked structures that are capable of releasing CyDs (cyclic compounds).

Discussion

In this study, CyD-containing polycatenanes were prepared by a facile one-pot method that is applicable to various fields. In particular, the prepared polycatenanes contained biodegradable disulfide bonds, making them promising supermolecules for biomaterials and drug-carrier applications.

Notably, although β-CyD polycatenanes and γ-CyD polycatenanes could be prepared, α-CyD polycatenane could not. Since α-CyD forms a polypseudorotaxane with the PEG moieties in HS–PEG–PPG–PEG–SH, whereas β-CyD and γ-CyD form polypseudorotaxanes with the PPG moieties[24,45], the α-CyD-based polypseudorotaxane probably does not undergo cyclization because of the decreased mobilities of the terminal thiol groups of HS–PEG–PPG–PEG–SH. Hence, future attempts to prepare α-CyD-based polycatenanes must consider the use of other axial molecules such as PPG–PEG–PPG.

The coverage and yield of γ-CyD polycatenane were higher and lower than those of β-CyD polycatenane, respectively (Table 1).

γ-CyD forms polypseudorotaxanes not only with PPG but also with PEG[9,13]. The threading of PEG chains of HS–PEG–PPG–PEG–SH onto γ-CyD likely resulted in high coverage of γ-CyD polycatenane. Moreover, it probably inhibited the cyclization of γ-CyD/HS–PEG–PPG–PEG–SH polypseudorotaxane, leading to the low yield of γ-CyD polycatenane.

The synthesized mono-β-CyD polycatenane and mono-γ-CyD polycatenane contain average 13.5 and 22.9 CyD molecules, respectively. In addition, di-polycatenanes and multi-polycatenanes contain many more CyD molecules. These results correspond to the largest number of cyclic components found in any radial polycatenane reported to date. Previously, the largest number of cyclic components in a discrete catenane was reported for a [7]catenane[27–29,46–48]. Only a few [*n*]catenanes with $n \geq 5$ have been reported[49], with most studies reporting CyD-based catenanes bearing a maximum of two CyDs ([3]catenane)[36,50]. Importantly, we believe that higher-order polycatenanes exhibit novel properties distinct from [2]- and [3]catenanes and are thus well suited to the development of advanced supramolecular structures and materials, such as molecular machines, molecular actuators, molecular switches, biomaterials, and drug carriers.

Interestingly, the mono-polycatenanes and di-polycatenanes showed low polydispersity index (M_w/M_n) (Table 2). Generally, β-CyD-threaded polyrotaxanes also show low polydispersity index (<1.10) probably due to homogeneous β-CyD units threaded into the polyrotaxanes[51–53]. Similarly, the number of β-CyD threaded in mono-polycatenanes and di-polycatenanes could be homogeneous, resulting in narrow polydispersity index.

This study introduced the one-pot syntheses and facile isolation of polycatenanes bearing more than 10 CyD units. Despite a number of reports on CyD-based polyrotaxanes[13,19,23], very little is known about CyD-based polycatenanes. In addition, few studies on radial polycatenanes have appeared since the first examples reported by Sauvage et al. in 1991[27]. We believe that the results reported herein are applicable to the development of advanced supramolecular structures and materials, including molecular machines, molecular actuators, molecular switches, biomaterials, and drug carriers.

Methods

Materials. CyDs were donated by Nihon Shokuhin Kako (Tokyo, Japan). PEG–PPG–PEG (Pluronic P123; M_w of PEG ≈ 880 × 2 Da, M_w of PPG ≈ 4060 Da) and HS–PEG–SH (M_w ≈ 8000 Da) were purchased from Sigma Chemicals (St. Louis, MO, USA). All other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

Preparation of HS-PEG-PPG-PEG-SH. Pluronic P123 (5.80 g), CDI (3.30 g), and triethylamine (152 mg) were dissolved in DMSO (20.0 mL), and the solution was stirred at room temperature for 24 h under nitrogen. Subsequently, cystamine dihydrochloride (4.50 g) in DMSO (50.0 mL) was added dropwise, followed by stirring at room temperature for 48 h. The reaction mixture was treated with dichloromethane (200 mL) and saturated sodium chloride solution (250 mL), and the aqueous layer was discarded. The organic layer was washed again with saturated sodium chloride solution (250 mL), and the aqueous layer was discarded; this process was repeated twice. The organic phase was evaporated, and the residue was lyophilized. The crude product (2.00 g) was dissolved in 35.0 mL of aqueous DTT (25.0 mg/mL), and the solution pH was adjusted to 7.0. After agitation for 48 h at room temperature under nitrogen, the pH of the solution was adjusted to 3.5, the sample was lyophilized, and the residue was dissolved in dichloromethane (60.0 mL). The organic phase was washed with saturated sodium chloride solution (30.0 mL), and the aqueous layer was discarded. This process was repeated twice, and the organic layer was evaporated to dryness. The product was lyophilized to afford HS–PEG–PPG–PEG–SH (3.30 g, 55.1%).

Attempted preparation of α-CyD-based polycatenanes. HS–PEG–SH (154 mg, M_w = 8000 Da) was dissolved in an aqueous solution of α-CyD (12.0 mL, 145 mg/mL). The mixture was then sonicated for 30 min and stirred at room temperature for 24 h. A hydrogen peroxide (H_2O_2) solution (120 μL, 30–32 w/v%) was added and the suspension was stirred at room temperature for 2 h. The resulting precipitate was collected by centrifugation (9000 rpm, 10 min), washed with water (35.0 mL), and dissolved in DMSO (10.0 mL). The solution was added dropwise to excess acetone (200 mL), and the precipitate was collected and washed with water (35.0 mL). After centrifugation, the precipitate was washed with water (35.0 mL) again, and dissolved in DMSO (10.0 mL). No precipitate was obtained after the adding to water (50.0 mL), as shown in Table 1.

Preparation of the β-CyD-based polycatenane. HS–PEG–PPG–PEG–SH (1.45 g) was dissolved in an aqueous solution of β-CyD (591 mL, 18.5 mg/mL). The mixture was then sonicated for 30 min and stirred at room temperature for 24 h. H_2O_2 (6.00 mL, 30–32 w/v%) was added and the suspension was stirred at room temperature for 2 h. The resulting precipitate was collected by centrifugation (9000 rpm, 10 min), washed with water (450 mL), and dissolved in DMSO (72.0 mL). The solution was added dropwise to excess acetone (420 mL), and the precipitate was collected and washed with water (420 mL). After centrifugation, the precipitate was washed with water (30.0 mL) again and collected by centrifugation. The resulting precipitate was lyophilized to afford the β-CyD-based polycatenane (611 mg, 11.6% yield).

Preparation of the γ-CyD-based polycatenane. HS–PEG–PPG–PEG–SH (24.5 mg) was dissolved in an aqueous solution of γ-CyD (10.0 mL, 190 mg/mL), and the mixture was sonicated for 30 min and stirred at room temperature for 24 h. H_2O_2 (100 μL, 30–32 w/v%) was added, and the suspension was stirred at room temperature for 2 h. The resulting precipitate was collected by centrifugation (9000 rpm, 10 min), washed with water (7.00 mL), and dissolved in DMSO (1.20 mL). The solution was added dropwise to excess acetone (7.00 mL), and the precipitate was collected and washed with water (7.00 mL). After centrifugation, the precipitate was re-dissolved in DMSO (1.00 mL). The solution was added dropwise to excess water (10.0 mL), and the precipitate was collected and washed with water (3.00 mL). After centrifugation, the resulting precipitate was lyophilized to give the γ-CyD-based polycatenane (2.3 mg, 1.6% yield).

Quantification of the thiol group content. HS–PEG–PPG–PEG–SH (600 mg) was dissolved in an aqueous solution of β-CyD (245 mL, 18.5 mg/mL). The mixture was then sonicated for 30 min and stirred at room temperature for 24 h. Different amounts of H_2O_2 (30–32 w/v%) were added to aliquots of the suspension such that the final H_2O_2 concentrations were 0.0001, 0.001, 0.005, 0.01, 0.05, 0.1, 1.0, and 2.0 v/v%, after which they were stirred at room temperature for 2 h. An aliquot of each resultant suspension (100 μL) was diluted with DMSO (900 μL) and 10.0 μL of Ellman's reagent (4.0 mg/mL of 5,5′-dithiobis(2-nitrobenzoic acid) in pH 7.4 phosphate-buffered saline (PBS)) was added. After incubation at room temperature for 10 min, an aliquot of the reaction mixture (750 μL) was diluted with PBS (750 μL, pH 7.4), and its absorbance was measured using a spectrophotometer (V-630, JASCO, Tokyo, Japan) at 360–550 nm.

Spectroscopy. Raman spectra were recorded using a RENISHAW inVia Raman microscope (New Mills, UK) under the following conditions: laser power = 100% (45 mW/line); excitation wavelength = 532 nm; spectral acquisition = 7979; exposure time = 5 s; cumulated number = 1; spatial resolution = 1 μm. Powder X-ray diffraction patterns were recorded on a Rigaku Ultima IV XRD system (Tokyo, Japan) using Ni-filtered Cu K_α radiation, a voltage of 40 kV, a current of 40 mA, a scanning speed of 5°/min, and a time constant of 2 s. AFM was performed using an AFM5100N/AFM5000 II instrument (Hitachi High-Tech Science Corporation, Tokyo, Japan) equipped with a SI-DF40P2 cantilever. ^{1}H-NMR spectra were recorded in deuterated DMSO at 25 °C on a JEOL JNM-ECP500 spectrometer (Tokyo, Japan) operating at 500 MHz. The proportions of CyD residues in the polycatenanes were determined by comparing the integrated signal intensities of the anomeric protons (4.8 ppm) of the CyDs with those of the PPG methylene protons (1.0 ppm).

GPC measurements. The polycatenane GPC profiles were obtained on an HLC-8120 system (Tosoh, Tokyo, Japan) under the following conditions: column = combination of TSKgel AW-4000 and AW-2500 columns (150 mm × 6 mm ID, Tosoh); elution solvent = 10 mM LiBr in DMSO; flow rate = 150 μL/min; temperature = 65 °C; detection = refractive index. The polydispersity index (M_w/M_n) was calculated from a calibration curve of PEG standards (Agilent Technologies, Wilmington, DE, USA). Samples (10.0 mg/mL) were dissolved in the elution solvent, and the solutions were filtered through a 0.22-μm polytetrafluoroethylene (PTFE) filter prior to analysis. To confirm the release of β-CyD from the polycatenane upon its reduction, the polycatenane (5.0 mg/mL) was mixed with a solution of DTT (25.0 mg/mL) or TCEP (2.9 mg/mL) in the elution solvent, followed by GPC.

Particle size measurements. The polycatenane solution in DMSO (100 μL, 1.0 mg/mL) was diluted with 900 μL of aqueous DTT (25.0 mg/mL), and particle sizes were measured on a Zetasizer Nano system (Malvern Instruments, Worcestershire, UK).

Release studies. A polycatenane sample (10.0 mg) was dispersed in 10.0 mL of aqueous DTT (25.0 mg/mL). After incubation at room temperature, a 1-mL sample

was collected and centrifuged (10,000 rpm, 3 min), and the concentration of β-CyD in the supernatant was determined by polarimetry (JASCO polarimeter P-2000, Tokyo, Japan).

Data availability

All data are available from the authors upon reasonable request.

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Author contributions

T.H., K.M., X.S., J.Z., and A.T. jointly carried out experiments and data analysis. K.M. and N.Y. helped with experiments and experimental design. T.H., H.A., and J.L. supervised the research and contributed to experimental design, manuscript writing, and revision.

Additional information

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