Threading α-Cyclodextrin through Poly[(*R,S*)-3-hydroxybutyrate] in Poly[(*R,S*)-3-hydroxybutyrate]−Poly(ethylene glycol)−Poly[(*R,S*)-3-hydroxybutyrate] Triblock Copolymers: Formation of Block-Selected Polypseudorotaxanes

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ABSTRACT: A series of polypseudorotaxanes were synthesized from α-cyclodextrin (α-CD) and poly[(*R,S*)-3-hydroxybutyrate]−poly(ethylene glycol)−poly[(*R,S*)-3-hydroxybutyrate] (PHB−PEG−PHB) triblock copolymers with flanking PHB blocks of different lengths and the middle PEG block of M_n 3000 Da. Formation of inclusion complexes was confirmed by X-ray diffraction data, with α-CD adopting channel-type crystalline structures. The ^{1}H NMR spectroscopy and thermogravimetric analysis results confirmed the presence of both host and guest molecules, and the compositions determined thereof from the two techniques were in good agreement. In the presence of excess α-CD, complexation stoichiometries between ethylene oxide units and α-CD for all polypseudorotaxanes were near the theoretical value of 2 despite the different lengths of PHB chains of the copolymers. Together with differential scanning calorimetry measurements where crystallization of the middle PEG block of the copolymers was completely absent while the glass transition of atactic PHB was detected, α-CD was thought to selectively cover the middle PEG block leaving telechelic PHB uncovered. The hypothesis was further substantiated by kinetic measurements; precipitation due to aggregation of the stable polypseudorotaxanes was slower with longer PHB chains. These findings demonstrated the successful threading of α-CD over the atactic PHB chain, which was previously thought to be impossible due to the mismatch in cross-sectional area. The study has highlighted the importance of block-selected molecular recognition of α-CD on PEG in the formation of stable polypseudorotaxanes of a block copolymer. The above revelations have interesting implications pertaining to design and synthesis of functional materials based on polypseudorotaxanes.

Introduction

Host−guest interactions of polymers and macrocycles have been the subject of extensive investigations. The endeavors have deepened our understanding on the intricacies of molecular recognition with the further dividend of establishing powerful platform for creation of new supramolecular self-assembled nanostructures.[1–6] Polypseudorotaxanes that are formed through threading of multiple cyclodextrin (CD) macrocycles along a polymeric axle, in particular, have demonstrated immense potential for biomedical applications, electronics, and polymer processing.[7–26] CDs are a class of naturally occurring cyclic oligosaccharides that mainly consist of 6, 7, or 8 D(+)-glucose units held together by α-1,4-linkages, namely α-, *β*-, and *γ*-CD. These toruslike molecules have a hydrophobic cavity of depth around 7 Å and internal diameters of 4.5, 7.0, and 8.5 Å with increasing sugar units.[27]

The threading of CDs onto polymeric chains is essentially an enthalpy-driven process, resulting from the favorable interactions between the polymeric guest and the hydrophobic cavity of CDs as well as formation of hydrogen bonding between adjacent threaded CDs. However, for threading to occur at all, the CDs' cavity must first be able to accommodate the polymer chain with certain cross-sectional area. This correlation of course will have implications on the thermodynamics and hence affect the stability of the resultant polypseudorotaxane. When the cavity is too large as compared to the cross-sectional area of polymer chain, host−guest interaction would be too weak or completely absent, while bulky moieties along polymer chain will impose substantial hindrance to the threading of macrocycles, in addition to perturbation on the hydrogen-bonding formation between neighboring hosts.[10] This interplay of forces was clearly embodied in the quantitative formation of polypseudorotaxanes by poly(ethylene glycol)s (PEGs) of various molecular weight and α-CD, but not with *β*-CD which has a larger internal diameter, as well as the absence of inclusion complexes (ICs) between α-CD and PEGs bearing bulky end groups.[11]

The mismatch between polymer cross-sectional area and CD cavity has been often cited as the main reason for failure of polypseudorotaxane formation.[28,29] While this is generally true, recent reports on the polypseudorotaxanes of poly[(ethylene oxide)-*ran*-(propylene oxide)] as well as poly[(propylene oxide)-*b*-(ethylene oxide)-*b*-(propylene oxide)] with α-CD shed new light on the importance of molecular recognition for the successful formation of polypseudorotaxanes.[30–32] The works presented the capability of propylene oxide moieties to penetrate the α-CD cavity, which was previously believed to be too small to accommodate the additional methyl group,[28] and that polypseudorotaxane formation was primarily driven by the favorable interaction of ethylene oxide units and α-CD. More recently, chiral recognition of α-CD was demonstrated to be operative in the preferential threading of poly(L-lactide) over poly(D-lactide), although both polymers have the same cross-sectional area.[33] Such findings present the possibility of preparing

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Table 1. Molecular Characteristics of PHB−PEG−PHB Triblock Copolymers Synthesized through Ring-Opening Polymerization of (*R,S*)-β-Butyrolactone with PEG-Macroinitiator

PHB−PEG−PHB copolymers[a]	M_n[b] (kDa)	PDI[b]	block length [M_n (kDa)]		PHB content (wt %)	
			PEG[c]	PHB[d]	NMR[e]	TGA[f]
H−E−H(12−68−12)	5.49	1.10	3.06	1.03	41.0	44.5
H−E−H(24−68−24)	6.88	1.08	3.06	2.06	56.5	59.2
H−E−H(30−68−30)	7.53	1.05	3.06	2.58	61.3	63.1

[a] PHB−PEG−PHB triblock copolymers are represented by the notation H−E−H($m-n-m$), where m and n denote number of 3-hydroxybutyrate and ethylene oxide repeating units, respectively. [b] Obtained from gel permeation chromatography (GPC) measurement. [c] Estimated from GPC and ^{1}H NMR spectroscopy. [d] Estimated from ^{1}H NMR spectroscopy. [e] Calculated based on intensity ratio of PHB methine proton (δ 5.2 ppm) and PEG methylene proton (δ 3.6 ppm). [f] Calculated from thermogravimetric analyses.

polypseudorotaxanes of novel architectures, which were previously thought to be inaccessible.

CD-based polypseudorotaxanes that consist of biodegradable polyester guests have gained much research impetus to date. As the constituents are biodegradable and biocompatible, such supramolecular entities are excellent candidates for smart biomaterials with easily tunable properties. Poly(lactide) (PLA),[34] poly(caprolactone) (PCL),[35,36] and poly(hydroxybutyrate) (PHB)[22,29,37] are some of the many polyesters found to be included by either one of the three CDs. Their copolymers, such as PCL−PEG−PCL,[38] PLLA−PEG−PLLA,[39] PEG−PHB−PEG,[24,40,41] and PCL−PLLA,[42] also form inclusion complexes with α-CD and exhibit interesting properties. In view of these exciting developments, we would like to explore the possibility of polypseudorotaxane formation from α-CD and triblock copolymers with poly[(*R,S*)-3-hydroxybutyrate]-*block*-poly(ethylene glycol)-*block*-poly[(*R,S*)-3-hydroxybutyrate] (PHB−PEG−PHB) chain topology, which were synthesized and characterized recently by our group.[43] The unique macromolecular architecture would allow us to investigate the possible threading of α-CD onto or over atactic PHB chain and perhaps elucidate the actual reason behind the inability of inclusion complex formation between atactic PHB and α-CD reported by Shuai et al.[29] Such understanding would then enable us to design and optimize material properties of the polypseudorotaxane.

Herein, we report the successful synthesis of a series of polypseudorotaxanes based on α-CD and PHB−PEG−PHB triblock copolymers with different PHB chain lengths, as evidenced by X-ray diffraction (XRD), ^{1}H NMR spectroscopy, differential scanning calorimetry (DSC), and kinetic study. The α-CD macrocycles were found to thread through the bulky atactic PHB chain, forming stable polypseudorotaxanes in reasonably high yields. Complexation stoichiometries of the polypseudorotaxanes, prepared with different polymer to α-CD ratios, were determined using ^{1}H NMR spectroscopy. Along with kinetic measurements and DSC data, they provided strong evidence for the selective coverage of middle PEG block in the polypseudorotaxanes by α-CD.

Experimental Section

Materials. Poly(ethylene glycol) (PEG) with number-averaged molecular weight (M_n) of ca. 3000 Da was obtained from Aldrich and purified by dissolving it in dichloromethane followed by precipitation in diethyl ether and drying under high vacuum at 40 °C for 48 h before use. M_n and polydispersity index (PDI = M_w/M_n) were found to be 2.99 kDa and 1.02 respectively. (*R,S*)-β-Butyrolactone (>95%) was supplied by Tokyo Kasei Inc. and purified by vacuum distillation from CaH_2 twice before use. 2,2,6,6-Tetramethyl-1-piperidinyloxy (TEMPO, 99%, Aldrich), α-cyclodextrin (>98%, Tokyo Kasei Inc.), sodium hypochlorite (NaClO, available chlorine ≥4%, Aldrich), hydrochloric acid (HCl, 1 N, Aldrich), 2-bromoethanol (95%, Aldrich), sodium bromide (NaBr, 99%, Alfa Aesar), sodium hydroxide (NaOH, 99%, Merck), sodium carbonate (Na_2CO_3, 99.9%, BDH), dimethyl sulfoxide (DMSO, anhydrous, ≥99.9%, Aldrich), ethanol (99.9%, Merck), diethyl ether (99.9%, J.T. Baker), hexane (99.8%, J.T. Baker), and chloroform (99.8%, Tedia) were used as received.

Synthesis of PHB−PEG−PHB Triblock Copolymers. The PHB−PEG−PHB triblock copolymers were obtained from anionic ring-opening polymerization of (*R,S*)-β-butyrolactone with a PEG-macroinitiator in DMSO at room temperature under dry N_2 atmosphere, while the macroinitiator was converted from commercially available hydroxyl-terminated PEG through a TEMPO-mediated oxidation, as reported previously.[43] Typically, 0.766 g of dried PEG-macroinitiator (M_n 3.06 kDa, 0.25 mmol) was first dissolved in 10 mL of DMSO followed by addition of 500 μL of β-butyrolactone (6.13 mmol) at room temperature. The reaction mixture was sampled periodically to determine monomer conversion using ^{1}H NMR spectroscopy, based on the intensity ratio of PHB methine proton (δ 5.1 ppm) and that of β-butyrolactone (δ 4.7 ppm). Upon reaching monomer conversion of >90%, the polymerization was quenched by adding excess 2-bromoethanol (100 μL, 1.42 mmol). DMSO was then removed by distillation, and the residue was dissolved in 30 mL of chloroform, filtered, and precipitated into 500 mL of *n*-hexane to afford the final copolymer. Yield: 1.02 g (70.%). GPC (THF): M_n = 7.53 kDa, PDI = 1.05. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26−1.31 (m, $-OCH(CH_3)CH_2CO_2-$ of PHB block), 2.43−2.63 (m, $-OCH(CH_3)CH_2CO_2-$ of PHB block), 3.46−3.70 (m, $-OCH_2CH_2O-$ of PEG block), 3.81 (t, J = 5 Hz, $-CO_2CH_2CH_2OH$ of end group), 4.10 (s, $-OCH_2CO_2-$ of PEG block), 4.21 (t, J = 5 Hz, $-CO_2CH_2CH_2OH$ of end group), 5.23−5.25 (m, $-OCH(CH_3)CH_2CO_2-$ of PHB block). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.8 ($-OCH(CH_3)CH_2CO_2-$ of PHB block), 40.8−40.9 ($-OCH(CH_3)CH_2CO_2-$ of PHB block), 60.8 ($-CO_2CH_2CH_2OH$ of end group), 66.3 ($-CO_2CH_2CH_2OH$ of end group), 67.7 ($-OCH(CH_3)CH_2CO_2-$ of PHB block), 68.6 ($-OCH_2CO_2-$ of PEG block), 70.6−70.9 ($-OCH_2CH_2O-$ of PEG block), 169.1−169.4 ($-OCH(CH_3)CH_2CO_2-$ of PHB block), 169.7 ($-CO_2CH_2CH_2OH$ of end group), 170.3 ($-OCH_2CO_2-$ of PEG block). IR (CaF_2): 3500, 2876, 1738, 1455, 1383, 1349, 1304, 1257, 1186, 1102, 1058, 963, 843 cm^{-1}. (Refer to Table S1 in Supporting Information for detail assignments of FT-IR absorption bands.)

Preparation of Polypseudorotaxanes. Polypseudorotaxanes of PHB−PEG−PHB triblock copolymers with three different PHB chain lengths (refer to Table 1 for molecular characteristics of copolymers) were prepared by vortexing and ultrasonicating the bulk polymers in 2 mL of saturated α-CD solution (0.145 g/mL) for not more than 10 min at room temperature and left standing for at least 2 days. Feed ratios of polymers were varied from 0.5 to 5 wt % as summarized in Table 2. The solutions gradually turned turbid with either eventual precipitation of polypseudorotaxane aggregates or formation of hydrogels. The precipitates were collected by ultracentrifugation and washed with a limited amount of water to remove unthreaded α-CD before being subjected to freeze-drying. Percentage yields of the polypseudorotaxanes were calculated on the basis of the amount of polymer used and corresponding number of α-CD threaded on each polymer chain as determined by ^{1}H NMR spectroscopy.

Molecular Characterizations. Gel permeation chromatography (GPC) measurements were carried out at 45 °C and at a flow rate of 0.2 mL/min, with a Shimadzu SCL-10A and LC-8A system equipped with two Phenogel 5μ 100 and 1000 Å columns (size: 300 × 4.6 mm) connected in series and a Shimadzu RID-10A refractive index detector. Tetrahydrofuran (THF) was selected as the mobile phase, and the system was calibrated with monodisperse poly(ethylene glycol) standards. ^{1}H NMR (400 MHz) and proton-

Table 2. Formulations for Polypseudorotaxane Preparation and Corresponding Yields

polypseudorotaxane[a]	copolymer used	amount of copolymer[b] (mg)	feed ratio (wt %)		yield[c] (mg)
			copolymer	α-CD	
PPR-H12-1	H−E−H(12−68−12)	12	0.5	12.6	63 (85.5%)
PPR-H12-2	H−E−H(12−68−12)	24	1.0	12.5	gel[d]
PPR-H12-3	H−E−H(12−68−12)	120	5.0	12.0	gel[d]
PPR-H24-1	H−E−H(24−68−24)	12	0.5	12.6	39 (63.4%)
PPR-H24-2	H−E−H(24−68−24)	24	1.0	12.5	gel[d]
PPR-H24-3	H−E−H(24−68−24)	120	5.0	12.0	gel[d]
PPR-H30-1	H−E−H(30−68−30)	12	0.5	12.6	28 (58.0%)
PPR-H30-2	H−E−H(30−68−30)	24	1.0	12.5	50 (66.3%)
PPR-H30-3	H−E−H(30−68−30)	120	5.0	12.0	220 (79.5%)

[a] Polypseudorotaxanes are named according to the notation PPR-H*m*-*x*, where *m* indicated the PHB chain length in terms of number of repeating unit and *x* the different formulation used within a copolymer series. [b] Copolymers were mixed with 2 mL of saturated α-CD solution (0.145 g/mL). [c] Percentage yield was calculated base on the amount of copolymer used and number of α-CD rings threaded on every polymer chain as determined from ^{1}H NMR spectroscopy. [d] Yields for gelled samples were not determined due to difficulty in removing unthreaded α-CD.

decoupled ^{13}C NMR (100 MHz) spectra were obtained on a Bruker AV-400 NMR spectrometer at room temperature and chemical shifts reported in ppm with reference to solvent peaks ($CHCl_3$: δ 7.26 for ^{1}H NMR and δ 77.2 for ^{13}C NMR; DMSO: δ 2.50 for ^{1}H NMR). Fourier transform infrared (FT-IR) spectra of the polymers coated on CaF_2 plate were recorded on a Bio-Rad 165 FT-IR spectrophotometer; 16 scans were signal-averaged with a resolution of 2 cm^{-1} at room temperature.

Thermal Analysis. DSC measurements were performed on a TA Instruments Q100 differential scanning calorimeter equipped with an autocool accessory and calibrated using indium. The following protocol was used for each sample: heating from room temperature to 150 °C at 5 °C min^{-1}, isothermal at 150 °C for 3 min, cooling from 150 to −80 °C at 5 °C min^{-1}, isothermal at −80 °C for 3 min and finally reheating from −80 to 150 °C at 5 °C min^{-1}. Data collected from second heating runs were analyzed. TGA were done on a TA Instruments SDT 2050 by heating the samples at a rate of 20 °C min^{-1} from room temperature to 800 °C in a dynamic nitrogen atmosphere (flow rate = 120 mL min^{-1}).

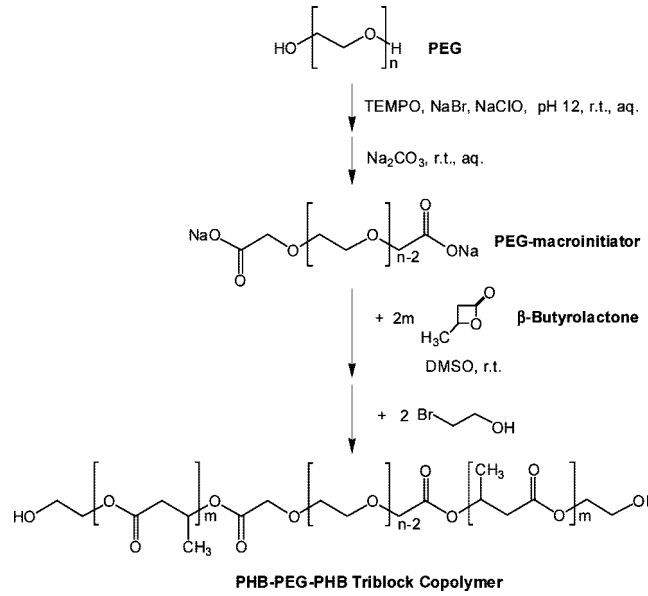
Wide-Angle X-ray Diffraction. XRD measurements were carried out by using a Bruker GADDS diffractometer with an area detector operating under Cu Kα (1.5418 Å) radiation (40 kV, 40 mA) at room temperature. Dried polypseudorotaxane samples were mounted directly onto a sample holder using double-sided adhesive tape while polymer samples were first deposited on glass slides before mounted onto the sample holder with double-sided adhesive tape. The samples were scanned from 5° to 40° (2θ).

Turbidity Measurements. Time-course absorbance measurements were performed on a Shimadzu UV-2501PC spectrophotometer at 25 °C. In general, 0.3 mL of polymer solution (33 mg/mL) and 2 mL of saturated α-CD solution were premixed and transferred to a cuvette with 1.0 cm cell length. The measurements were started one minute after premixing at wavelength 600 nm. The absorbance values were referenced against deionized water.

Results and Discussion

Molecular Characteristics of PHB−PEG−PHB Triblock Copolymers. The synthetic scheme for PHB−PEG−PHB triblock copolymers is presented in Scheme 1. Commercially available PEG with telechelic hydroxyl groups was first converted to the corresponding PEG−bis(sodium carboxylate) macroinitiator via a TEMPO-mediated oxidation and subsequent neutralization with Na_2CO_3. The process has allowed convenient and efficient preparation of pure macroinitiator that is required for a controlled growth of PHB chains from both ends of the PEG-macroinitiator. The attachment of two PHB chains on the macroinitiator was accomplished through ring-opening polymerization of β-butyrolactone in DMSO under ambient inert conditions. As shown by Juzwa et al.,[44] the novelty of this approach lies in the ability to obtain PHB with desired molecular weight of narrow distribution, without the need of activation using a toxic crown ether. This is highly relevant for biomaterial synthesis as potential contamination from toxic catalysts or activators can be avoided. Additional advantages of this synthetic strategy include the option to decorate the end group of PHB chain using various capping agents, with good fidelity, as well as the ability to regulate chain microstructure by tuning the relative amount of β-butyrolactone stereoisomers in the monomer feed.

Scheme 1. Synthesis of Poly[(*R,S*)-3-hydroxybutyrate]-*block*-poly(ethylene glycol)-*block*-poly[(*R,S*)-3-hydroxybutyrate] (PHB−PEG−PHB) Triblock Copolymers by Ring-Opening Polymerization of (*R,S*)-β-Butyrolactone with PEG-Macroinitiator at Room Temperature in DMSO[a]



[a] TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy; DMSO = dimethyl sulfoxide.

Three PHB−PEG−PHB triblock copolymers were used for the preparation of polypseudorotaxanes, and they were synthesized from PEG-macroinitiator with M_n of 3.06 kDa, each having two flanking PHB chains of 12, 24, and 30 repeating units, respectively, and were named respectively as H−E−H(12−68−12), H−E−H(24−68−24), and H−E−H(30−68−30). The triblock architecture of PHB−PEG−PHBs has been established collectively by ^{1}H NMR, ^{13}C NMR, and FT-IR spectroscopies, GPC, and TGA. Molecular characteristics of the copolymers are given in Table 1. Essentially, ^{1}H NMR, ^{13}C NMR, and FT-IR spectroscopies revealed the coexistence of PEG and PHB blocks in copolymers, free from starting materials. The relative amount of PHB present as well as molecular weight of copolymers as estimated from ^{1}H NMR spectroscopy corroborated well with TGA and GPC data and were almost the

theoretical values. High purity of the triblock copolymers was characterized by narrow molecular weight distribution from GPC measurements and negligible crotonate signal detected by ^{1}H NMR spectroscopy. This information is important to ensure that the resultant polypseudorotaxanes are strictly the product of triblock copolymers with telechelic PHB chains, and α-CD macrocycles must thread through the PHB chains before any inclusion complex can be formed. On a further note, Shuai et al. have shown the effect of PHB chain microstructures on formation of inclusion complex with CDs.[29] To this end, we concluded from ^{13}C NMR and FT-IR spectroscopies as well as DSC study that the PHB chains in the copolymers were atactic, and hence should α-CD macrocycles form polypseudorotaxanes with the copolymers, their cavity must be able to accommodate the larger cross-sectional area of the atactic PHB chain than isotactic ones. (Refer to Figures S1 and S2 in Supporting Information for ^{1}H NMR and ^{13}C NMR spectra of triblock copolymer.)

Preparation and Formation of Polypseudorotaxanes. Nine polypseudorotaxanes were prepared according to the copolymer to α-CD feed ratios tabulated in Table 2. The changes in copolymer to α-CD ratios were done by varying the amount of copolymer used while keeping the volume of saturated α-CD solution constant at 2 mL. These variations in formulation aimed at the systematic study on the effect of PHB chain length on threading of α-CD as well as to probe the phenomenological differences arising from changes in copolymer to α-CD ratios and from thereon elucidate the mode of complexation of α-CD on the triblock copolymers. The 0.5 wt % of copolymer usage represented the lower end of copolymer to α-CD ratio, where the amount of α-CD is more than required to obtain complete coverage along the copolymer backbone (assuming maximum complexation ratios of ethylene oxide (EO):α-CD = 2 and 3-hydroxybutyrate (HB):α-CD = 1.5),[11,29] while 1.0 and 5.0 wt % copolymer loading corresponded to around 83−93% and 16−19% coverage, respectively. The polypseudorotaxanes were named according to the notation PPR-H*m*-*x*, where *m* represents the PHB chain length of copolymer used in terms of repeating unit and *x* the different formulation within the same copolymer series with 1, 2, and 3 representing copolymer loading of 0.5, 1.0, and 5.0 wt %, respectively.

When saturated α-CD solution was added to the copolymers, the solutions gradually turned turbid upon ultrasonication, followed by precipitation or gelation over time. The copolymers were found to remain intact after ultrasonication according to GPC and ^{1}H NMR evaluations, and the amounts of precipitates were all greater than the corresponding copolymer, suggesting that the precipitates collected were not due to any impurities. Based on the complexation stoichiometry as determined from ^{1}H NMR spectroscopy, the yield of washed precipitates was moderate to high, ranging from 58% to 86%. These phenomenological changes provided the first indication of the formation of inclusion complexes. For more conclusive evidence, the washed precipitates were dried and subjected to XRD analyses. Gelled samples, on the other hand, were freeze-dried and analyzed with XRD unwashed. XRD traces of all the precipitates and dried hydrogels are shown in Figures 1 and 2, respectively. Strong diffraction peaks centered around $2\theta = 19.8°$ ($d = 4.49$ Å), which is characteristics of α-CD with channel-type crystalline phase,[45,46] were present in all samples, and their diffraction patterns were all similar to that of α-CD/PEG polypseudorotaxane but very different from those of pristine α-CD and triblock copolymers, e.g., H−E−H(12−68−12). The formation of such a columnar crystalline phase was induced by the threading of polymer chain through the cavities of multiple α-CD rings[10] and hence confirmed the formation of polypseudorotaxanes through inclusion complexation of α-CDs with the

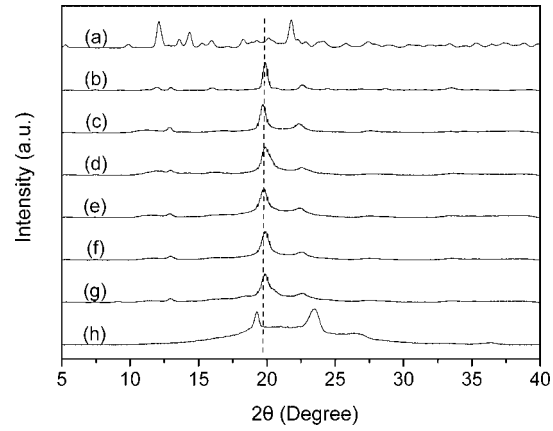


Figure 1. Room temperature X-ray diffraction traces of (a) pristine α-CD, (b) αCD/PEG(M_n 2.99 kDa) inclusion complex, (c) PPR-H12-1, (d) PPR-H24-1, (e) PPR-H30-1, (f) PPR-H30-2, (g) PPR-H30-3, and (h) triblock copolymer H−E−H(12−68−12). The intense diffraction peaks for all the inclusion complexes are found at around $2\theta = 19.8°$, as marked by the dashed vertical line.

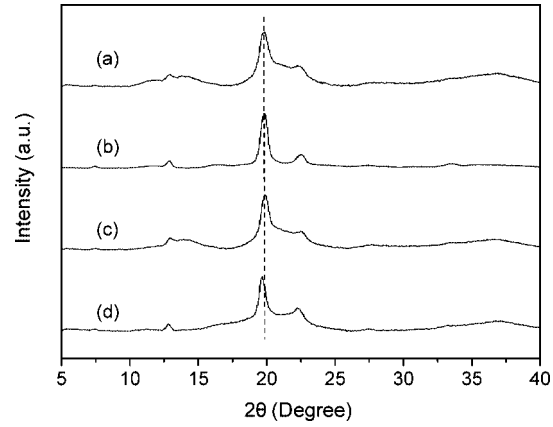


Figure 2. X-ray diffraction traces of dried powders recovered from lyophilized hydrogels of (a) PPR-H12-2, (b) PPR-H12-3, (c) PPR-H24-2, and (d) PPR-H24-3. The intense diffraction peaks for all the inclusion complexes are found at around $2\theta = 19.8°$, as marked by the dashed vertical line.

copolymer chains. However, their diffraction peaks appeared broadened in comparison to those of the α-CD/PEG inclusion complex, and this reflected lower crystallinity which might be caused by amorphous PHB present. On the other hand, the threading of α-CD significantly altered solid state properties of the guest copolymer chain, preventing formation of the crystalline PEG phase that is otherwise signified by two diffraction peaks at $2\theta = 19.3°$ and 23.5°, which were apparent in uncomplexed copolymers.

A representative ^{1}H NMR spectrum belonging to PPR-H30-3 is shown in Figure 3 along with corresponding peak assignments. Signals from both the α-CD host and guest copolymer H−E−H(30−68−30) can be clearly identified, and the composition of each purified polypseudorotaxane and the average number of α-CD threaded on each copolymer chain were estimated by comparing the signal intensity of H1 of α-CD at δ 4.7 ppm to that of PHB methine proton at δ 5.1 ppm. The calculated values are listed in Table 3, and they should be interpreted with the knowledge that these values correspond to polypseudorotaxanes that were obtained after long reaction times (>2 days) and washed once with a limited amount of water.

At a copolymer loading of 0.5 wt % where the amount of α-CD was sufficient for complete chain coverage, we had complete dissolution of copolymers except H−E−H(30−68−30).

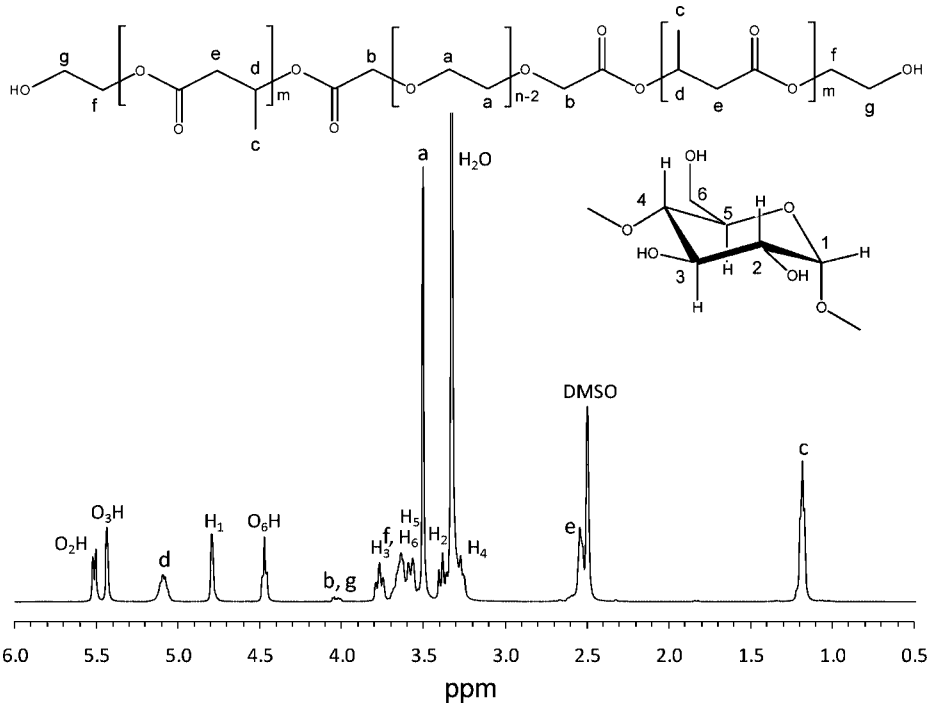


Figure 3. 400 MHz ^{1}H NMR spectrum of polypseudorotaxane PPR-H30-3 in deuterated dimethyl sulfoxide (DMSO-d_6) at room temperature with corresponding peak assignments.

Table 3. Complexation Stoichiometries of Purified Polypseudorotaxanes, Their α-CD Content, and Thermal Properties

					α-CD content (wt %)				
polypseudorotaxane	α-CD/copolymer chain[a]	EO:α-CD[b]	HB:α-CD[c]	(EO + HB):α-CD[d]	NMR[a]	TGA[e]	T_{onset} (°C)[f]	T_d (°C)[g]	T_g (°C)[h]
PPR-H12-1	29.0	2.34	0.83	3.17	84.8	79.7	212 (177)	305 (234)	n.d.[i]
PPR-H24-1	29.2	2.33	1.64	3.97	80.0	75.1	217 (177)	282 (228)	1.8
PPR-H30-1	23.4	2.91	2.56	5.47	73.6	68.2	225 (191)	292 (242)	0.6
PPR-H30-2	16.6	4.10	3.61	7.71	66.4	61.1	245 (191)	297 (242)	−4.4
PPR-H30-3	10.1	6.73	5.94	12.70	54.7	49.2	247 (191)	296 (242)	−9.9

[a] Number of α-CD rings threaded on each copolymer chain, as determined from ratio between integrals of α-CD H1 signal at δ 4.7 ppm and PHB methine proton at δ 5.1 ppm. [b] Calculated by dividing number of α-CD ring per copolymer chain to the number of ethylene oxide repeating unit. [c] Calculated by dividing number of α-CD ring per copolymer chain to the number of 3-hydroxybutyrate repeating unit. [d] Calculated by dividing number of α-CD ring per copolymer chain to the total number of repeating unit. [e] Calculated from thermogravimetric analyses. [f] Temperature for onset of thermal degradation. Values given in parentheses are onset temperatures of corresponding triblock copolymers. [g] Degradation temperature is defined as temperature where 10% mass loss occurred. Values given in parentheses are degradation temperatures of corresponding triblock copolymers. [h] Determined from second heating run of differential scanning calorimetry. [i] Not detected.

A comparable amount of α-CD threaded on H−E−H(12−68−12) and H−E−H(24−68−24) but lesser on H−E−H(30−68−30). The lesser amount of threaded α-CD on H−E−H(30−68−30) might be due to longer PHB chain that posed greater hindrance to the threading of α-CD and increased hydrophobicity of bulk H−E−H(30−68−30), which led to difficult dispersion of copolymer in saturated α-CD solution. When correlated to the almost identical amount of α-CD threaded on H−E−H(12−68−12) and H−E−H(24−68−24) despite a doubling in PHB chain length, the dispersion of H−E−H(30−68−30) in aqueous solution could be the dominant factor for lower α-CD threading at 0.5 wt % copolymer loading. These data, however, do not negate the threading obstacle imposed by the PHB chain as the associated time scale eclipsed the time frame required to distinguish differences in α-CD threading onto or over PHB of different chain length.

When the copolymer amount was increased to 1.0 and 5.0 wt % as in the case of PPR-H30-2 and PPR-H30-3, the number of α-CD threaded in every copolymer chain decreased steadily. This again can be attributed to the poor dispersibility of H−E−H(30−68−30). However, as the α-CD to copolymer ratio decreased, the equilibrium of inclusion complex formation itself was affected and hence posed as another important factor for lesser number of α-CD threaded. Although quantitative data on the number of α-CD threaded per copolymer chain for PPR-H12-2, PPR-H12-3, PPR-H24-2, and PPR-H24-3 are not available, the formation of a hydrogel that is widely known to be due to reduced coverage of polymer chain[47] suggests their trend should follow that of PPR-H30 series.

The distribution of α-CD along the copolymer can be generalized into the following three scenarios: (a) random distribution on both PEG and PHB blocks, (b) α-CD rings mainly located at flanking PHB blocks due to some specific interaction or merely because of insufficient energy to reach middle PEG segment, and (c) due to favorable interaction, the threaded α-CD rings may preferentially reside on the middle PEG, as pictured in Figure 4. In terms of complexation stoichiometries, when α-CD rings are distributed randomly, the molar ratio of total repeating unit to α-CD would lie in the range of 1.5−2 for complete chain coverage. However, if α-CD rings selectively cover the PHB segment under similar reaction conditions as the previous scenario, a complexation stoichiometry approaching 1.5 between HB and α-CD is anticipated along with an increment in amount of α-CD threaded when the PHB chain length increases. In the final scenario, one would expect a complexation stoichiometry of EO:α-CD approaching 2 regardless of PHB chain length so long as sufficient amount of

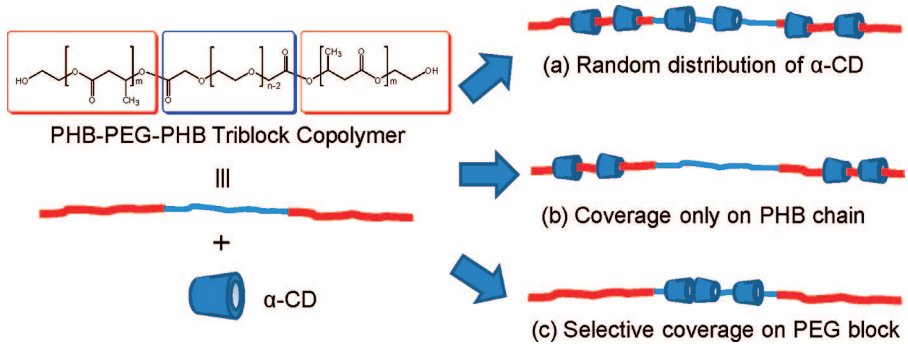


Figure 4. Schematic representations on the possible mode of α-CD distribution along PHB−PEG−PHB triblock copolymers.

α-CD is present to drive the complexation forward and cover the whole PEG block.

Complexation stoichiometries of purified polypseudorotaxanes, prepared under high α-CD to copolymer ratio, were consistent with the third scenario. Molar ratios of EO to α-CD for PPR-H12-1, PPR-H24-1, and PPR-H30-1 are in between 2 and 3, while their HB to α-CD ratios as well as total repeating unit to α-CD ratios do not conform to any of the scenarios described. This strongly suggests that the middle PEG block of the copolymers were selectively included by α-CD while PHB chains at both sides remain largely uncovered. The value larger than 2 might result from the hindrance of PHB chain on α-CD threading. The data do not, however, exclude the possibility of α-CD covering the PHB chain during preparation of polypseudorotaxanes. Nevertheless, should this occur, the selectivity of α-CD for PEG block is still evident from the complexation stoichiometries of the purified polypseudorotaxanes.

Thermal Analyses. Threading of α-CD onto the copolymer chains has significant effect on the crystallization behavior of the guest copolymer as seen from XRD study. The thermal properties of purified polypseudorotaxanes were investigated using DSC, and their second heating curves as well as those of guest triblock copolymers are separately shown in parts a and b of Figure 5. All three triblock copolymers exhibited melting endotherm around 20−40 °C. However, these melting endotherms disappeared after threading of α-CD. This suggested that crystallization of PEG block has been inhibited entirely by α-CD that formed a sheath covering the segment, which is consistent with XRD results. On the other hand, glass transition temperatures (T_gs) were detected on PPR-H24-1, PPR-H30-1, PPR-H30-2, and PPR-H30-3. No T_g was observed for PPR-H12-1 as the parent copolymer itself had only a weak glass transition. Triblock copolymers showed single glass transitions from the miscible amorphous PEG and PHB segments. As such, the temperature at which the transition occurred depended on the relative amount of each component according to the Gordon−Taylor equation[48] and lie within the typical T_gs of PEG and atactic PHB, which are around −50 and 0 °C,[49] respectively. Thus, when lesser amorphous PEG is present, the T_g of the triblock copolymer will be at a higher temperature tilting toward that of atactic PHB homopolymer and vice versa. In comparison with their guest copolymers, the polypseudorotaxanes have higher T_gs. Moreover, by correlating with ^{1}H NMR data, we can see a proportional relationship between T_g and α-CD coverage as evident from the PPR-H30 series. Such correlation is possible when α-CD rings cover predominantly PEG segments and hence restrict the mixing with the PHB chains, increasing the T_g of copolymer toward that of PHB homopolymer. When α-CD coverage is lower, the extent of PEG mixing with PHB would be greater and hence push the copolymer T_g lower. A selective coverage of PHB chain would lead to a reverse trend. Consequently, the DSC data further confirm the hypothesis in which the middle PEG block was preferentially

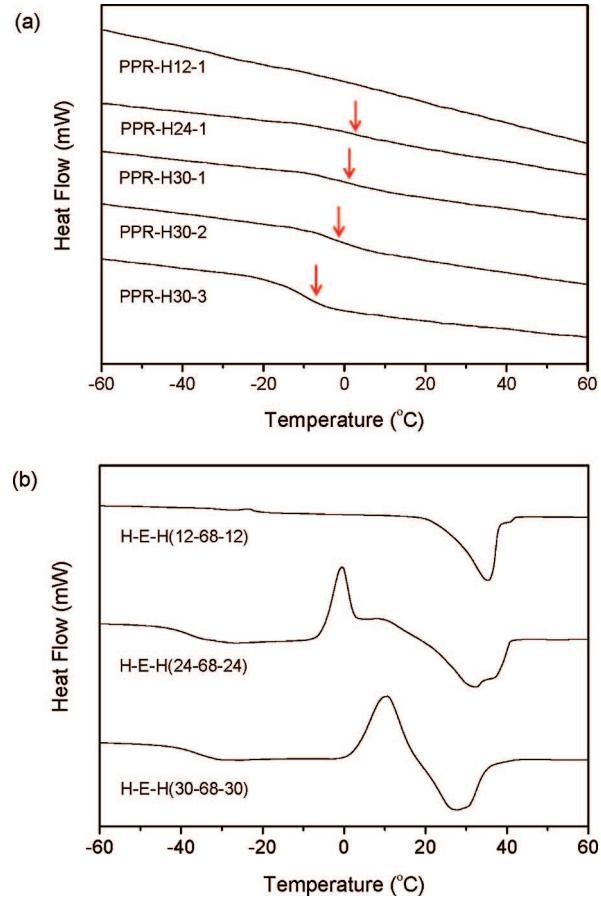


Figure 5. Differential scanning calorimetry thermograms of (a) polypseudorotaxanes with arrows indicating respective glass transitions and (b) PHB−PEG−PHB triblock copolymers. Thermograms presented are all second heating curves.

included by α-CD and the amorphous PHB remained largely uncovered.

The thermal stabilities of the polypseudorotaxanes were evaluated using TGA, and a representative three-step degradation profile of PPR-H30-3 is shown in Figure 6 along with its precursors. Albeit poor resolution between the first two steps of degradation, the first one can still be attributed to the loss of PHB segment, followed by α-CD and finally the PEG segment based on graphical comparison with those of the precursors. Moreover, as the degradation step of PEG can be fairly resolved from the other steps of mass loss, it was used to estimate copolymer content in the polypseudorotaxanes. Taking experimental error into account, the calculated values, listed in Table 2, corroborate fairly well with ^{1}H NMR estimation. From the quantitative data presented in Table 3, marked improvements in thermal stability were observed for all the purified polyp-

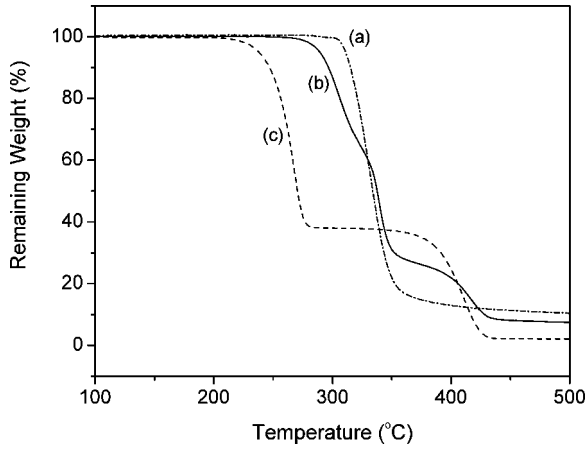


Figure 6. Thermogravimetric analyses on (a) pristine α-CD, (b) polypseudorotaxane PPR-H30-3, and (c) triblock copolymer H−E−H(30−68−30).

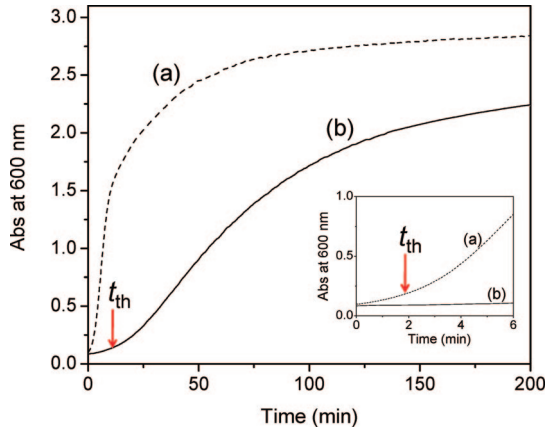


Figure 7. Time-course absorbance measurements at 600 nm for threading of α-CD onto triblock copolymers (a) H−E−H(12−68−12) and (b) H−E−H(24−68−24). Threading time (t_{th}) for both systems are indicated by an arrow.

seudorotaxanes as compared to their respective guest copolymers, in terms of later onset of degradation (T_{onset}) and higher degradation temperature (T_d). The observations were in line with other reports pertaining to improved thermal stability of polypseudorotaxane with respect to their guest polymers.[32,40]

Complexation Kinetics. According to model proposed by Ceccato et al.,[50] the course of polypseudorotaxane formation from PEG and α-CD encompassed five elementary steps that begins with diffusion of host and guest molecules, followed by initial threading of α-CD by PEG ends, sliding of α-CD into middle of PEG, dethreading of α-CD, and finally precipitation of aggregated polypseudorotaxanes. The evolution of turbidity during inclusion complexation is associated with the formation of a stable polypseudorotaxane that leads to aggregates of highly crystalline nature. On the basis of these premises, the threading kinetics of α-CD through H−E−H(12−68−12) and H−E−H-(24−68−24) were compared by measuring turbidity development in mixtures with a large excess of α-CD. The time-course absorbance measurements shown in Figure 7 were characterized by an initial region of constantly low absorbance and a subsequent regime with a sharp increase in absorbance before plateauing off at maximum values. Threading time (t_{th}) is defined as the onset of sharp absorbance increase, and it was found to be dependent on PHB chain lengths, with t_{th} of H−E−H(12−68−12) around 2 min while that of H−E−H(24−68−24) approached 12 min. The results again implied the selective coverage of α-CD on the middle PEG block as more time was needed to thread pass long PHB chains in order to form stable polypseudorotaxanes that eventually aggregated to give insoluble crystalline particles.

Conclusions

New polypseudorotaxanes have been successfully synthesized using PHB−PEG−PHB triblock copolymers with different PHB chain length and α-CD. The formation of inclusion complexes, which exhibited improved thermal stability over the precursors, was ascertained from XRD measurements where α-CD of the polypseudorotaxanes adopted the characteristics channel-type crystalline structure. The presence of both host α-CD and guest copolymers in polypseudorotaxanes was confirmed by ^{1}H NMR spectroscopy, and α-CD contents were found to range from 54 to 85 wt %, close to TGA estimations. On the basis of the complexation stoichiometries of 2−3 EO repeating units to one α-CD for all polypseudorotaxanes prepared in an excess of α-CD, DSC analyses where PEG crystallization was shown to be completely inhibited by α-CD complexation while telechelic amorphous PHB chains displayed T_g approximating that of amorphous PHB homopolymer and turbidity measurements where copolymer with shorter PHB chains formed stable polypseudorotaxane at a faster rate, we conclude that the middle PEG block of copolymers were selectively included over the flanking PHB chains.

The block selected inclusion of α-CD on the middle PEG block of PHB−PEG−PHB triblock copolymers is a demonstration on the penetration of atactic PHB through α-CD that has been considered to be impossible on account of polymer cross section. Additionally, although threading onto atactic PHB occurred, α-CD macrocycles did not stay on the chain but proceed further to the middle PEG block, indicating that certain form of molecular recognition was operative and served as an important driving force for polypseudorotaxane formation. Moreover, the lack of favorable interaction between α-CD and PHB also explains why α-CD did not form inclusion complexes with atactic PHB homopolymer. These findings illustrate that the often thought cross-sectional mismatch may not be the actual reason for nonformation of inclusion complexes. In fact, a certain degree of flexibility of α-CD macrocycles may be present and thus broadens our horizon in designing polypseudorotaxane-based functional materials, in terms of materials selection and design parameters.

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Supporting Information Available: Representative ^{1}H and ^{13}C NMR spectra as well as FT-IR absorption band assignments of the PHB−PEG−PHB triblock copolymers. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Lehn, J. M. *Supramolecular Chemistry: Concepts and Perspectives*; VCH: Weinheim, 1995.
(2) Huang, F. H.; Gibson, H. W. *Prog. Polym. Sci.* **2005**, *30*, 982–1018.
(3) Chen, L.; Zhu, X. Y.; Yan, D. Y.; Chen, Y.; Chen, Q.; Yao, Y. F. *Angew. Chem., Int. Ed.* **2006**, *45*, 87–90.
(4) Raymo, F. M.; Stoddart, J. F. *Chem. Rev.* **1999**, *99*, 1643–1663.
(5) Oku, T.; Furusho, Y.; Takata, T. *Angew. Chem., Int. Ed.* **2004**, *43*, 966–969.
(6) Whang, D.; Jeon, Y. M.; Heo, J.; Kim, K. *J. Am. Chem. Soc.* **1996**, *118*, 11333–11334.
(7) Harada, A.; Li, J.; Kamachi, M. *Nature (London)* **1992**, *356*, 325–327.
(8) Wenz, G.; Keller, B. *Angew. Chem., Int. Ed.* **1992**, *31*, 197–199.
(9) Wenz, G. *Angew. Chem., Int. Ed.* **1994**, *33*, 803–822.

(10) Wenz, G.; Han, B. H.; Muller, A. *Chem. Rev.* **2006**, *106*, 782–817.
(11) Harada, A.; Li, J.; Kamachi, M. *Macromolecules* **1993**, *26*, 5698−5703.
(12) Harada, A.; Li, J.; Kamachi, M. *Nature (London)* **1993**, *364*, 516–518.
(13) Harada, A.; Li, J.; Kamachi, M. *Nature (London)* **1994**, *370*, 126–128.
(14) Okumura, Y.; Ito, K. *Adv. Mater.* **2001**, *13*, 485–487.
(15) Ooya, T.; Choi, H. S.; Yamashita, A.; Yui, N.; Sugaya, Y.; Kano, A.; Maruyama, A.; Akita, H.; Ito, R.; Kogure, K.; Harashima, H. *J. Am. Chem. Soc.* **2006**, *128*, 3852–3853.
(16) Sakai, T.; Murayama, H.; Nagano, S.; Takeoka, Y.; Kidowaki, M.; Ito, K.; Seki, T. *Adv. Mater.* **2007**, *19*, 2023–2025.
(17) Yui, N.; Ooya, T. *Chem.—Eur. J.* **2006**, *12*, 6730–6737.
(18) Rusa, C. C.; Wei, M.; Bullions, T. A.; Shuai, X. T.; Uyar, T.; Tonelli, A. E. *Polym. Adv. Technol.* **2005**, *16*, 269–275.
(19) Uyar, T.; Oguz, G.; Tonelli, A. E.; Hacaloglu, J. *Polym. Degrad. Stab.* **2006**, *91*, 2471–2481.
(20) Lu, J.; Mirau, P. A.; Tonelli, A. E. *Prog. Polym. Sci.* **2002**, *27*, 357–401.
(21) Dong, T.; Kai, W.; Pan, P.; Cao, A.; Inoue, Y. *Macromolecules* **2007**, *40*, 7244–7251.
(22) He, Y.; Inoue, Y. *Biomacromolecules* **2003**, *4*, 1865–1867.
(23) Li, J.; Yang, C.; Li, H.; Wang, X.; Goh, S.; Ding, J.; Wang, D.; Leong, K. *Adv. Mater.* **2006**, *18*, 2969–2974.
(24) Li, J.; Li, X.; Ni, X.; Wang, X.; Li, H.; Leong, K. *Biomaterials* **2006**, *27*, 4132–4140.
(25) Cacialli, F.; Wilson, J. S.; Michels, J. J.; Daniel, C.; Silva, C.; Friend, R. H.; Severin, N.; Samori, P.; Rabe, J. P.; O'Connell, M. J.; Taylor, P. N.; Anderson, H. L. *Nat. Mater.* **2002**, *1*, 160–164.
(26) Taylor, P. N.; O'Connell, M. J.; McNeill, L. A.; Hall, M. J.; Aplin, R. T.; Anderson, H. L. *Angew. Chem., Int. Ed* **2000**, *39*, 3456–3460.
(27) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: Berlin, 1978.
(28) Harada, A.; Okada, M.; Li, J.; Kamachi, M. *Macromolecules* **1995**, *28*, 8406–8411.
(29) Shuai, X. T.; Probeni, F. E.; Wei, M.; Bullions, T.; Tonelli, A. E. *Macromolecules* **2002***35*, 3778–3780.
(30) Li, J.; Li, X.; Toh, K.; Ni, X.; Zhou, Z.; Leong, K. *Macromolecules* **2001**, *34*, 8829–8831.
(31) Li, J.; Ni, X.; Leong, K. *Angew. Chem., Int. Ed.* **2003**, *42*, 69–72.
(32) Li, J.; Ni, X.; Zhou, Z.; Leong, K. *J. Am. Chem. Soc.* **2003**, *125*, 1788–1795.
(33) Ohya, Y.; Takamido, S.; Nagahama, K.; Ouchi, T.; Ooya, T.; Katoono, R.; Yui, N. *Macromolecules* **2007**, *40*, 6441–6444.
(34) Rusa, C. C.; Tonelli, A. E. *Macromolecules* **2000**, *33*, 5321–5324.
(35) Kawaguchi, Y.; Nishiyama, T.; Okada, M.; Kamachi, M.; Harada, A. *Macromolecules* **2000**, *33*, 4472–4477.
(36) Huang, L.; Allen, E.; Tonelli, A. E. *Polymer* **1998**, *39*, 4857–4865.
(37) Shuai, X. T.; Porbeni, F. E.; Wei, M.; Bullions, T.; Tonelli, A. E. *Macromolecules* **2002**, *35*, 3126–3132.
(38) Lu, J.; Shin, I. D.; Nojima, S.; Tonelli, A. E. *Polymer* **2000**, *41*, 5871–5883.
(39) Choi, H. S.; Ooya, T.; Sasaki, S.; Yui, N.; Ohya, Y.; Nakai, T.; Ouchi, T. *Macromolecules* **2003**, *36*, 9313–9318.
(40) Li, X.; Li, J.; Leong, K. *Macromolecules* **2003**, *36*, 1209–1214.
(41) Li, X.; Li, J.; Leong, K. *Polymer* **2004**, *45*, 6845–6851.
(42) Wei, M.; Shuai, X. T.; Tonelli, A. E. *Biomacromolecules* **2003**, *4*, 783–792.
(43) Liu, K. L.; Goh, S. H.; Li, J. *Polymer* **2008**, *49*, 732–741.
(44) Juzwa, M.; Jedlinski, Z. *Macromolecules* **2006**, *39*, 4627–4630.
(45) McMullan, R. K.; Saenger, W.; Fayos, J.; Mootz, D. *Carbohydr. Res.* **1973**, *31*, 37–46.
(46) Takeo, K.; Kuge, T. *Agric. Biol. Chem.* **1970**, *34*, 1787–1794.
(47) Li, J.; Harada, A.; Kamachi, M. *Polym. J.* **1994**, *26*, 1019–1026.
(48) Gordon, M.; Taylor, J. S. *J. Appl. Chem.* **1952**, *2*, 493.
(49) Kemnitzer, J. E.; McCarthy, S. P.; Gross, R. A. *Macromolecules* **1992**, *25*, 5927–5934.
(50) Ceccato, M.; LoNostro, P.; Baglioni, P. *Langmuir* **1997**, *13*, 2436–2439.

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