Recognition by Lipases of ω-Hydroxyl Macroinitiators for Diblock Copolymer Synthesis

Ajay Kumar and Richard A. Gross*

Department of Chemistry and Chemical Engineering, NSF-IUCR Center for Biocatalysis and Bioprocessing of Macromolecules, Polytechnic University, Six Metrotech Center, Brooklyn, New York 11201

Yunbing Wang and Marc A. Hillmyer

Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455

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ABSTRACT: The mono-terminal hydroxyl groups of polybutadiene of varying chain length were studied as sites for initiation of lactone ring-opening polymerization by lipase catalysis. Novozyme-435 (immobilized *Candida antarctica* lipase B) was an effective catalyst for this acylation. Monohydroxyl-terminated polybutadiene with M_n of 2.6, 10, and 19 kg/mol efficiently initiated ω-pentadecalactone (PDL) and ϵ-caprolactone (CL) polymerization in toluene at 55 °C to give polybutadiene-*b*-polypentadecalactone and polybutadiene-*b*-polycaprolactone, respectively. For example, monohydroxyl-terminated polybutadiene with M_n 2.6 kg/mol initiated PDL ring-opening polymerization (1:25, OH:PDL) and formed a product with M_n 9.6 kg/mol in 19 h with 17 mol % chains initiated by water and >90% initiator efficiency. This method circumvented previously published chemical methods that required a metal catalyst and strict exclusion of moisture. A fractionation method was developed to separate the oligomers and to reduce the homopolymer contamination in the diblock copolymer. In situ NMR experiments were used to monitor the initiation of CL ring-opening polymerization with PBD-OH. Characterization of polymer was performed using ^{1}H and ^{13}C NMR spectroscopies and GPC.

Introduction

Enzymes, harvested from living organisms, are finding increasing use as catalysts for polymer synthesis in vitro.[1−3] Some enzymes have attracted interest for use in polymer synthesis since they (i) are derived from renewable resources, (ii) function well at moderate temperatures, (iii) are active in bulk reactions, organic media, and at various interfaces, (iv) catalyze reactions with high enantio- and regioselectivity, (v) are recyclable, and (vi) can replace other catalysts that are highly reactive, are toxic, and/or introduce heavy metals into products. Also, lipases do not require the exclusion of air and water when functioning as catalysts for polyester synthesis.[4,5] In this paper, lipases were studied as catalysts for the synthesis of block copolymers.

Model block copolymers of narrow molecular weight distribution generally involve sequential monomer addition using synthetically accessible living or controlled polymerization techniques.[6−8] The range of block copolymers can be increased through the incorporation of mechanistically incompatible monomers into a single block copolymer. This can be accomplished by the transformation of one type of active center to another. For example, polydiene−polylactide block copolymer syntheses were reported using a sequential combination of living anionic polymerization and controlled coordination insertion polymerization.[9,10] The syntheses required the preparation of hydroxyl-terminated polydiene chains and reaction of the ω-hydroxyl group with triethylaluminum. This gave aluminum alkoxide-activated terminal species that acted as macroinitiators for the polymerization of lactide. A difficulty with this strategy is that trialkylaluminum is highly reactive with water. Thus, strict precautions to exclude moisture must be taken.

A well-known method employed to toughen brittle polymers is to incorporate a discrete phase of rubber particles into the rigid polymer matrix. HIPS, ABS, and SBS are representative examples of toughened polymeric materials that are prepared using this technique.[11] For these systems, block copolymers are needed to increase the compatibility between phases. This paper describes lipase-catalyzed ring-opening polymerization used to build a polyester chain segment from the hydroxyl terminal group of low-T_g amorphous rubber-containing blocks. Of particular interest was to use lipase catalysis to prepare chain segments consisting of poly(ω-pentadecalactone), poly(PDL). Except for one recent report with a highly water-sensitive catalyst system,[12] traditional chemical catalysts have poor activity for the polymerization of ω-pentadecalactone (PDL). In contrast, lipase-catalyzed (e.g., using *Candida antarctica* lipase B) routes to poly(PDL) have great promise.[13−15]

Recently, we reported on the solid-state properties of poly(PDL).[16] This polymer has a glass transition at −30 °C and is crystalline with melting around 100 °C. Poly(PDL) shows good thermal stability, with a main TGA weight loss (50%) centered at 425 °C. The mechanical properties of poly(PDL) are typical of a hard−tough material, with an elastic modulus and yield parameters comparable with those of low-density polyethylene.

This paper describes the use of lipases to grow polyester chain segments from the terminal monohydroxyl group of synthetic polymers. Focus areas include the effects of the monohydroxyl macroinitiator concentration and its chain length on block copolymer formation. Since low levels of water were present in the reactions, both water and the monohydroxyl group of the macroinitiators were expected to compete as poten-

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Table 1. Reaction Profile of Polybutadiene Initiated PDL/CL Polymerization

reaction	monomer/ initiator	initial[a] $M_n \times 10^{-3}$	initial[b,e] $M_n \times 10^{-3}$	[M]/[I]	time (h)	% yield[c] MeOH ppt	% PDL/CL content[d]	% homo-poly(PDL)[a]	product[b] $M_n \times 10^{-3}$	PDI[b]
1	PDL/PBD-OH	2.6	4.7	50	1.2	NR	55	24	13.8	1.96
2	PDL/PBD-OH	2.6	4.7	50	4.5	NR	55	30	15.8	1.74
3	PDL/PBD-OH	2.6	4.7	50	23	86	52	<5	15.5	1.66
4	PDL/PBD-OH	2.6	4.7	25	19	90	31	12	9.6	1.66
5	PDL/PBD-OH	10.0	15.2	25	19	92	11	20	21.2	1.16
6	PDL/PBD-OH	19.0	34.7	50	1.2	NR	17	27	39.8	1.10
7	PDL/PBD-OH	19.0	34.7	50	4.5	NR	17	33	42.7	1.12
8	PDL/PBD-OH	19.0	34.7	50	23	87	12	6	42.7	1.12
9	PDL/PBD-OH	19.0	34.7	25	19	94	6	20	38.0	1.17

[a] From ^{1}H NMR. [b] From GPC in $CHCl_3$. [c] Monomer conversion in all the cases was >95%. NR = nonfractionated samples as were withdrawn in small amounts as aliquots. [d] Total mol % of PDL/CL contribution in the product. [e] PBDOH in entree 1−4, 10 had PDI 1.03 and [OH]/[*sec*-Bu] 0.99, PBDOH in entree 5 had PDI 1.02 and [OH]/[*sec*-Bu] 0.98 and PBDOH in entree 6−9 had PDI 1.02 and [OH]/[*sec*-Bu] 1.0.

tial nucleophiles or acceptors of enzyme-activated acyl groups. If the monohydroxyl groups of the macroinitiators compete effectively with water, then lipase catalysis will favor the formation of diblocks relative to water-initiated carboxyl-terminated polyester chains. As macroinitiators, monohydroxyl-terminated polybutadiene (PBD-OH) of varying molecular weight was used.

Experimental Section

A. Material and Methods. ω-Pentadecalactone (99.5%) and toluene were purchased from Aldrich Chemical Co. Inc. ϵ-Caprolactone (99%) was a gift from Union Carbide Co. ϵ-Caprolactone and toluene were dried over calcium hydride and distilled under a nitrogen atmosphere. Coulomat A and Coulomat C were purchased from EM Science. Immobilized lipase from C*andida antarctica* (Novozyme-435) was gift from Novo Nordisk Bio-industries, Inc. Enzymes was dried over P_2O_5 (0.1 mmHg, 25 °C, 24 h) prior to their use. Water content of the enzyme was measured using an EM Science coulometric titrator.

General Procedure for Preparation of Polyolefin with Terminal Hydroxy Group. A detailed description of the polymerization apparatus and procedure for the synthesis of hydroxy-terminated polybutadienes has been previously reported.[10]

General Procedure for Lipase-Catalyzed Initiation of Lactones Using Polymeric Chains. Novozyme-435 (0.074 g) dried in a vacuum desiccator (0.1 mmHg, 25 °C, 24 h) was transferred under a nitrogen atmosphere into an oven-dried 50 mL round-bottomed flask containing hydroxy-terminated polybutadiene (M_n 2.6 kg/mol, 1.3 g, 0.5 mmol of OH) and PDL/CL (6.025 g, 25 mmol of PDL). The vials were stoppered with rubber septa and sealed with Teflon tape. Toluene (37 mL) was subsequently added under nitrogen using a syringe. The vial was then placed into a constant temperature (55 °C) oil bath for 23 h. Reactions 1−3 and 6−8 (Table 1) were performed for varying times (1.2−23 h), with PDL to PBD-OH 50:1 (mol/mol) and with PBD-OH segments with M_n values of 2.6 kg/mol (reactions 1−3) and 19 kg/mol (reactions 6−8). To monitor the progress of reactions with time, a small quantity (1% of the total) of the reaction mixture from reactions 1, 2, 6, and 7 were withdrawn with a syringe, diluted with $CHCl_3$, and filtered through a Gelman acrodisc 13CR PTFE 0.45 μm filter. The solvents were removed prior to product NMR and GPC analysis. Monomer conversions (percent) were monitored after 1.2 and 4.5 h by NMR of the dried aliquots (NMR spectroscopy). To terminate reactions 3 and 8 (after 23 h), the enzyme was removed by adding excess chloroform and filtration to remove the insoluble enzyme (glass-fritted filter, medium pore porosity). The insoluble portion was washed several times with hot chloroform. The chloroform in the filtrate was completely removed by rotary evaporation, and the polymer was analyzed to determine the monomer conversion. It was then precipitated by adding it into methanol. The precipitate was isolated by filtration and then dried in a vacuum oven (0.1 mmHg, 50 °C, 24 h). The product (above example) was isolated in 86% yield with average molecular weight (M_n 15.5 kg/mol and PDI 1.66) and <5% water-initiated chains.

Polybutadiene−Polypentadecalactone/Polycaprolactone Characterization. NMR spectra of poly(BD-*b*-PDL) ($CDCl_3$) $-[CH_2-CH=CH-CH_2/CH_2=CH-CH=CH_2(\sim 90/10)-O]_n-[O=C-CH_2^b-CH_2^c-\{-CH_2^d-CH_2^d-\}_5-CH_2^c-CH_2^a-O-]_x$-($M_n$15.5 kg/mol and PDI 1.66) were as follows: ^{1}H NMR ($CDCl_3$): δ 5.4 (m, $-CH_2-CH=CH-CH_2-$ and $-CH_2=CH-CH-$), 4.9 (m, $-CH_2=CH-CH-$), 4.07 (t, J 6.5 Hz, CH_2^aO), 3.62 (t, J 6.5 Hz, CH_2OH), 2.29 (t, J 7.5 Hz, CH_2^bCO), 2.0 (b, $-CH_2-CH=CH-CH_2-$ and $CH_2=CH-CH-$), 1.65 and 1.3−1.2 (brs, $CH_2^{c,d}$, $CH_2=CH-C(R)H-CH_2-$ and includes intensity from butyl group), 0.9 (m, $-CH_3$, resonance from terminal secondary butyl group). The resonances at 3.62 and 2.29 for terminal CH_2 attached to OH/COOH groups were shifted to 4.35 and 2.88 when a drop of oxalyl chloride was added to the product solution in the NMR tube and recorded.

The ^{13}C NMR spectroscopy resonances reported below are for the majority cis 4,1 microstructure of PBD-OH. The regio- and geometrical isomers of PBD not listed were present in low amounts. ($CDCl_3$) δ 173.9 ($COCH_2$), 129−130 ($-CH=CH-$), 64.4 (CH_2^aO and $O=COCH_2-$PBD), 62.9 ($HOCH_2$), 34.4 ($OCOCH_2^b$), 32.6 and 27.3 ($-CH_2-CH=CH-CH_2-$) 29.6−29.1, 28.6, 25.9, and 25.0 (all other carbons of poly(PDL) ppm.

NMR spectra of poly(BD-*b*-CL) ($CDCl_3$) $-[CH_2-CH=CH-CH_2/CH_2=CH-CH=CH_2(\sim 90/10)-O]_n-[O=C-CH_2^d-(CH_2)_2^c-CH_2^b-CH_2^a-O-]_x$ (M_n 5.9 × 10^3 g/mol and PDI 1.3) was as follows: ^{1}H NMR ($CDCl_3$): δ 5.4 (m, $-CH_2-CH=CH-CH_2-$ and $-CH_2=CH-CH-$), 4.9 (m, $-CH_2=CH-CH-$), 4.05 (t, J 6.5 Hz, CH_2^aO), 3.61 (t, J 6.5 Hz, CH_2OH), 2.30 (t, J 7.5 Hz, CH_2^dCO), 2.0 (b, $-CH_2-CH=CH-CH_2-$ and $CH_2=CH-CH-$), 1.65 and 1.4−1.3 (brs, $CH_2^{b,c}$), 1.2 (b, $CH_2=CH-C(R)H-CH_2-$, and includes intensity from butyl group), 0.88 (m, $-CH_3$, resonance from terminal *sec*-butyl group). The resonances at 3.61 and 2.30 for terminal CH_2 attached to OH/COOH groups were shifted to 4.35 and 2.88 when a drop of oxalyl chloride was added to the product solution in the NMR tube and recorded. ^{13}C NMR ($CDCl_3$): δ 173.4 ($COCH_2$), 142.5, 129−130 ($-CH=CH-$), 114.1 ($CH_2=CH$), 64.0 (CH_2^aO and $O=COCH_2-$PBD), 62.9 ($HOCH_2^a$), 34.2 ($OCOCH_2^d$), 32.6 and 27.3 ($-CH_2-CH=CH-CH_2-$) 28.2 (CH_2^b), 25.4 and 24.5 ppm (CH_2^c).

Fractionation of Reaction Mixtures That Contained Poly(butadiene-*b*-pentadecalactone), Poly(BD-PDL), and Poly(PDL). To better understand the details of reactions between PBD-OH and PDL, fractionation of the product 4, Table 1 was performed. The reaction product (2.5 g) obtained by reacting PBD-OH (2.6 kg/mol) and PDL (1:25 OH: PDL) for 19 h was dissolved in a minimum amount of $CHCl_3$ (1.5 mL) and poured into methanol (100 mL). The methanol insoluble fraction (2.25 g) was filtered and dried in a vacuum oven (entry 1, Table 2). The MeOH soluble portion was rotatory evaporated to completely remove the organic solvents and was analyzed by NMR. Fractionation of the methanol-insoluble portion was further performed based on the relative solubility of the products in THF. PBD-OH is highly soluble in THF, whereas poly(PDL) and poly(BD-*b*-PDL), after only a few PDL

Table 2. Fractionation of PBD-*b*-31 mol % Poly(PDL) Obtained after 19 h Using PBDOH (2.6 kg/mol, NMR) and PDL in 1:25 OH:PDL

entry	[PDL]/[PBD][a]	% wt fraction	% water initiated[c]	av DP PDL chain[a]	product $M_n \times 10^3$ *a*	product $M_n \times 10^3$ *d*	PDI[d]
MeOH ppt fraction	31/69	100	12	21	7.6	9.6	1.66
THF-soluble fraction	14/86	40[b]	9	5	3.8	6.1	1.18
THF-insoluble fraction	43/57	60[b]	<5	35	11.0	12.6	1.57

[a] By NMR experiment. [b] Isolated yield with respect to methanol fraction. [c] Oxalyl chloride derivatization in the NMR experiments. [d] GPC in $CHCl_3$.

units, are sparingly soluble. A 10-fold excess of cold tetrahydrofuran (THF) was added to the methanol precipitated product and stirred for 5 min. The THF insoluble fraction (1.35 g) was filtered and dried in a vacuum oven. The THF-soluble portion was evaporated to dryness on a rotatory evaporator. This fractionation procedure was not applicable to PBDOH (19K) initiated polymeric products due to its increased solubility in THF. THF soluble and insoluble fractions were analyzed for their composition using NMR spectroscopy, and the results are tabulated in Table 2.

B. Instrumental Methods. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Instruments Inc. DPX300 spectrometer at 300 and 75.13 MHz, respectively. The chemical shifts in parts per million (ppm) for ^{1}H and ^{13}C NMR spectra were referenced relative to tetramethylsilane (TMS) as an internal reference at 0.00.

Molecular weights were determined by gel permeation chromatography (GPC) using a Waters HPLC system equipped with a model 510 pump, a Waters model 717 autosampler, a model 410 refractive index detector, and a model T-50/T-60 detector of Viscotek Corp. with 500, 10^3, 10^4, and 10^5 Å Ultrastyragel columns in series. Trisec GPC software version 3 was used for calculations. Chloroform was used as the eluent at a flow rate of 1.0 mL/min at 25 °C. Sample concentrations of 0.2% w/v and injection volumes of 100 μL were used. Molecular weights were determined on the basis of a conventional calibration curve generated by narrow molecular weight polystyrene standards obtained from Aldrich Chemical Co.

Initial reaction water contents (wt % water) were measured using an Aqua star C 3000 titrator with Coulomat A and Coulomat C from EMscience. The water wt/wt in reaction mixtures were determined by stirring 53 mg of Novozyme-435 and 2.7 mL of toluene in coulomat A in a closed septum container designed in the instrument and titrating it against coulomat C by the instrument. The total water content (wt/wt) in the reactions was ~1.5%.

Results and Discussion

The anionic polymerization of polybutadiene followed by the reaction with ethylene oxide is an established method for the preparation of hydroxy-terminated polybutadiene in high yields, with nearly quantitative functionalization (i.e., one hydroxy group per chain) and with narrow molecular weight distribution.[17,18] We prepared and characterized a variety of these samples.[10] The number-average molecular weights (M_n), regio- and geometrical isomerism, and end-group structure were analyzed by NMR spectroscopy. Polybutadiene, obtained by the above methods, has mostly 1,4-cis isomers.[10]

The primary hydroxyl groups in small molecules such as butanol,[19] ethylglucoside,[20] (4-hydroxyphenyl)ethanol,[21] and others[22] are nucleophiles or initiators for the ring-opening polymerization of ϵ-caprolactone. Serine present in the catalytic active site of the lipases is believed to form an acyl enzyme-activated monomer (EAM).[19] This EAM can react with a propagating macromolecular chain end to further increase the chain length. Alternatively, the EAM can react with a low molar mass initiator to form ester-terminated (R−O−[C═O]−CH_2−, R is initiator) chains.[19] Also, lipases actively catalyze the cleavage of intrachain ester groups

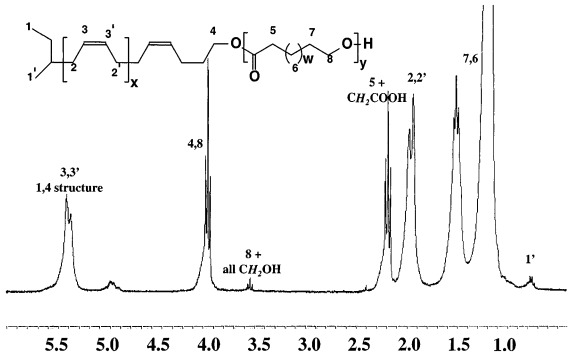
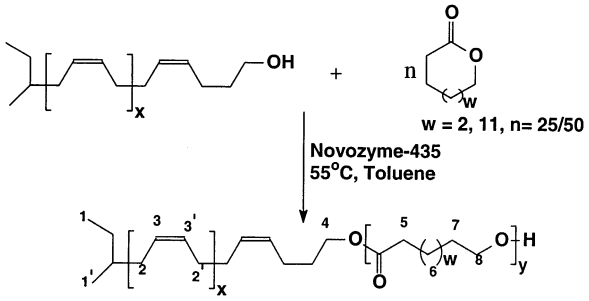


Figure 1. ^{1}H NMR spectra of polybutadiene-*b*-31 mol % polypentadecalactone.

Scheme 1. Biocatalytic Route to Polybutadiene-*b*-polyester Synthesis



of nonbranched aliphatic polyesters to give enzyme-activated chain segments (EACS).[23] The resulting EACS can react with a terminal hydroxyl group of another macromolecule to form an intrachain ester group. Another possible scenario is the initiation of PDL or CL chain growth by H_2O. This will result in HOOC-terminated polyester chains. These carboxyl terminal chains can remain as such or may react with PBD-OH with the elimination of water (condensation) to form PBD-polyester block copolymers.

Considering the above, the ability of monohydroxyl-terminated polymeric chains of polybutadiene to function as nucleophiles for chain initiation or transacylation reactions (Scheme 1) was assessed. In contrast to polyester chains, PBD-OH does not have ester groups and, therefore, is restricted to acylation reactions only at the chain terminal unit. The reactions were catalyzed by Novozyme-435, at 55 °C, in toluene, with PDL and CL as monomers. Representative results for a series of reactions between PBD-OH with either PDL or CL are shown in Table 1.

Proton (^{1}H) and carbon (^{13}C) NMR were used to analyze the products formed by reactions between PBD-OH and PDL. The ^{1}H NMR signals characteristic of polybutadiene and poly(PDL) are shown in Figure 1. The vinyl proton resonance of PBD-OH and the methylene region of poly(PDL) were well-separated and appeared at δ 5.4 and 4.07, respectively. The CH_2−

O(C═O)− protons of the PBD-OH unit at the poly(PBD-*b*-PDL) junction ester linkage should appear ∼δ 4.1. Unfortunately, this signal was not well resolved from intrachain poly(PDL) CH_2−O(C═O)− protons. To confirm that PBD-OH and poly(PDL) blocks are indeed joined by an ester link and to assess the extent of water-initiated poly(PDL) chains, the products were treated with an excess of oxalyl chloride and then analyzed by ^{1}H NMR. This resulted in a downfield shift in the signal at δ 3.62 (CH_2OH end group) to δ 4.35. Also, modification with oxalyl chloride resulted in a new signal at δ 2.88. The signals at δ 4.35 were assigned to all oxalyl chloride modified hydroxyl-terminal CH_2−OH protons of poly(PDL), poly(BD-*b*-PDL), and traces of unreacted PBD. The signals at δ 2.88 were assigned to oxalyl chloride modified poly(PDL) CH_2−(C═O)−OH groups that arise from chain initiation or transacylation reactions with water (see Supporting Information, Figure S-1). The ratio of the signal intensities at δ 2.88 and δ 4.35 was used to determine the percent of poly(PDL) chains with terminal carboxylic acid versus those with a PBD terminal chain, assuming a negligible amount of unreacted PBD-OH. These results were used to determine the percent of carboxyl-terminated homopoly(PDL) chains formed (Table 1).

To better understand the products from reactions between PBD-OH and PDL, fractionation of the product from reaction 4 was performed (for methods see Experimental Section). Based on ^{1}H NMR analysis, reaction 4 (1:25 hydroxyl of PBD-OH to PDL unit), conducted for 19 h at 55 °C, gave >95% PDL conversion. The isolated yield of the product after methanol precipitation was 90%. For all fractionated PDL/PBD-OH reaction products described in Table 1 (see reactions 3−5 and 8−9), the methanol soluble product was <12% of the total product. Analysis of the methanol-soluble portion by NMR showed the presence of cyclic polyPDL, PBD-OH, and oligomeric poly(PDL). Analysis by ^{1}H NMR of the methanol-insoluble fraction of reaction 4 showed that the ratio of PBD-OH to poly(PDL) repeat units was 69/31 mol/mol. Furthermore, GPC analysis of this fraction gave values of M_n and PDI (M_w/M_n) of 9.6 kg/mol and 1.66, respectively (Table 2). The methanol-insoluble portion from reaction 4 was fractionation based on the relative solubility of the products in THF (see Experimental Section). Comparison of the THF-soluble and -insoluble fractions showed that they have 14 and 43 mol % of PDL repeat units and represent 40 and 60 wt % of the methanol-insoluble material, respectively (Table 2). Also, NMR analysis, after a drop of oxalyl chloride addition, showed that the majority of HOOC-poly(PDL) was of low molecular weight (∼4 units) and was found in the THF-soluble fraction. The fraction of total HOOC-poly(PDL) chains found in the THF-soluble fraction was >82%. Thus, the THF-soluble fraction contains HOOC-poly(PDL), unreacted PBD-OH (signal at δ 62.5 in the ^{13}C NMR spectrum), and poly(BD-*b*-PDL) with short PDL chains. In contrast, the THF-insoluble fraction is almost free of contaminant HOOC-poly(PDL) (<5%) and PBD-OH homopolymers (no signal corresponding to PBD-CH_2OH in the ^{13}C NMR spectrum) and, therefore, consists almost entirely of poly(BD-*b*-PDL) chains. The GPC traces of reactants and products from the fractionation procedure are shown in Figure 2. Comparison of the PDI values for the THF-soluble vs the THF-insoluble fractions shows that the latter has a higher value (Table 2). This follows

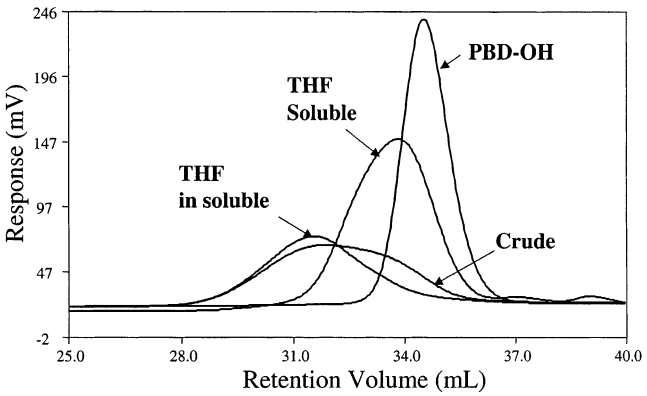


Figure 2. GPC profile for the fractionation of PBD-*b*-31 mol % poly(PDL).

from PDL chain segments within block copolymers that will shift the PDI value toward 2.0. The relative GPC peak intensities for equivalent weights of PBD-OH (34K, GPC) and poly(PDL) (60K, GPC) were 2.72/1. Thus, the refractive index of poly(PDL) and the eluent more closely match. This results in a disproportionately low sensitivity or response within GPC traces to contributions of poly(PDL) homopolymer or PDL-containing chain segments. Thus, the reported GPC values tend to underestimate the presence of HOOC-poly(PDL), the block lengths of poly(PDL) in copolymers, and also block copolymer PDI values.

Representative results for Novozyme-435-catalyzed PDL ring-opening polymerizations using PBD-OH macroinitiators are compiled in Table 1. For all reactions described in Table 1, monomer conversion for reaction times ≥ 1.2 h for PDL was >95%. Furthermore, as described above, fractionation of products by methanol precipitation gave low levels of methanol-soluble product (<12%). Thus, changes in product molecular weight due to methanol precipitation are assumed negligible in the discussion below.

Novozyme-435-catalyzed PDL polymerizations using PBD-OH M_n 2.6 kg/mol as the macroinitiator showed only a small increase in M_n from 1.2 to 4.5 h. An increase in the reaction time from 4.5 to 23 h resulted in no further change in M_n. The percentage of total chains that are homo-poly(PDL) decreased from 30% to 13% (<5% after MeOH precipitation) for reactions 2 and 3, respectively. We believe this is due to condensation reactions that occur slowly between unreacted PBD-OH and carboxyl terminal poly(PDL) chains. The decrease in dispersity from reaction 1 to 2 is due to the increased PBD-OH consumption (∼40−80%). The percent consumption of PBD-OH-initiated ring-opening polymerization of CL is discussed later in the text using in-situ NMR experiments and was assumed to be similar for PDL ring-opening polymerization.

Reactions 3 and 4 differed only by small changes in the reaction time and in the PDL/PBD-OH ratio (50:1 and 25:1, respectively) (Table 1). Relative to reaction 3, the reaction 4 product had a lower M_n but a much larger fraction of carboxyl terminal poly(PDL) chains. The decrease in M_n by lowering the monomer-to-macroinitiator ratio is expected and mirrors what was seen previously for lipase-catalyzed polymerizations with low molar mass nucleophiles.[19] PDL polymerizations was also performed using PBD-OH M_n 10 kg/mol as the macroinitiator (reaction 5, Table 1). When compared to the results with PBD-OH M_n 2.6 kg/mol for 19 h, a small

increase in the percent homo-poly(PDL), product M_n, and decrease in the polymer PDI were observed.

Encouraged by the above results, Novozyme-435-catalyzed initiation of PDL with 19 kg/mol PBD-OH was studied. The average degree of polymerization of 19 kg/mol PBD-OH was 351. Thus, the probability of encountering a terminal hydroxyl group relative to a butadiene repeat unit was small. Nevertheless, Novozyme-435 recognized the terminal hydroxyl groups of 19 kg/mol PBD-OH and catalyzed ester formation with these end groups. By using a ratio of 19 kg/mol PBD-OH to PDL of 50:1 mol/mol, by 1.2 h, a product with M_n of 39.8 and PDI of 1.1 was formed. By increasing the reaction time from 1.2 to 4.5 h, the polymer M_n (39.8K−42.7K) increased. The signal for the PBD-OH and poly(PDL) CH_2OH end groups had one peak each out of their triplet signal resolved by ^1H NMR (δ 3.7 and 3.6, respectively). Similar resolution was observed for their oxalyl chloride derivatized products. The ratio of their integrals was used to analyze the percent of carboxyl-terminated homo-poly(PDL) chains that were formed. The percent homo-poly(PDL) chains formed in reactions 6 and 7 was slightly larger when compared to reactions 1 and 2 (27 and 33 vs 24 and 30, respectively; see Table 1). These differences in the percent of homo-poly(PDL) formed may reflect a very small change in the efficiency of 2.6 and 19.0 kg/mol PBD-OH chains to function as macroinitiators for block copolymer formation. Nevertheless, study of the results in Table 1 shows that PBD-OH hydroxyl terminal groups have a high affinity for the active site of *Candida antarctica* lipase B that is hardly effected as the PBD-OH chain length was increased from 2.6 to 10 and 19 kg/mol. Furthermore, the GPC profile showed unimodal distribution for the products for reactions 6−9 and inspection of their PDI values showed that they were between 1.10 and 1.17. These low PDI values are explained by a combination of factors that include (i) the low dispersity of the starting PBD-OH initiator, (ii) the masking effect of PBD-OH in GPC due to the higher RI response factor for PBD-OH relative to PDL (see above), and (iii) the low molar ratio in the polymerization of PDL to PBD-OH repeat units (6−17 mol % for reactions 6−9).

To better understand the relationships between the reaction time, monomer conversion, and macroinitiator consumption, the reaction was run in the NMR tube that allowed in situ monitoring of both the percent monomer conversion and the percent esterification of PBD-OH. CL was used in place of PDL since the signals for the monomer and polymer were better resolved for CL in *d*-toluene then PDL. PBD-OH (2.6K) was used instead of their analogue of higher molar mass to avoid high viscosity and decreased PBD-OH end-group signal in the NMR tube. The NMR experiment was run in *d*-toluene (5 times vol/wt excess relative to monomer), PBD-OH to CL ratio of 1:25 mol/mol, at 55 °C, with shaking at 5−7 min intervals. The ratios between NMR signals at δ 3.94 and 3.64, and at δ 3.50 and 3.46, were used to calculate the percent monomer conversion and percent PBD-OH consumption, respectively (see Supporting Information Figure S-2). The profiles of percent CL conversion and PBD-OH consumption as a function of time are shown in Figure 3. The conversion of CL reached 70% and 90% in 100 and 300 min, respectively. Furthermore, esterification of PBD-OH terminal groups reached 65 and >85% in 100 and 250 min, respectively. Thus, even with the poor mixing during the NMR experiments, the consumption of both CL and PBD-OH was rapid and occurred concurrently.

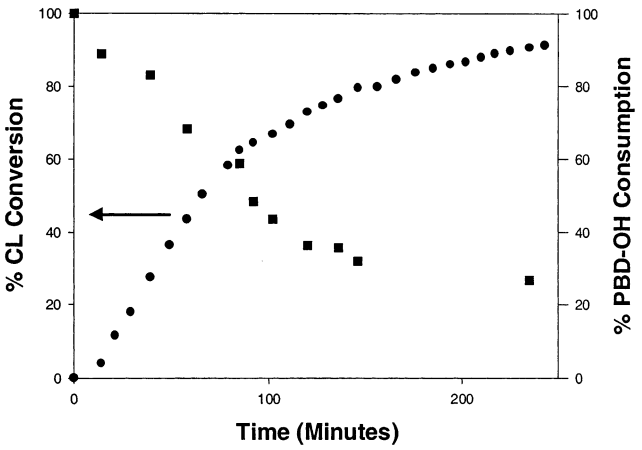


Figure 3. Reaction profile of polybutadiene (2.6 kg/mol, NMR) as initiator for CL ring-opening polymerization.

Conclusions

An enzymatic method was developed for polyester-*co*-polybutadiene diblock copolymer synthesis. The method consists of using a polydiene macromer with a terminal primary hydroxyl group to initiate the ring-opening polymerization of lactones under mild conditions. Polybutadiene macromers of varying chain length (2.6−19 kg/mol) were all active initiators for the ring-opening polymerization of lactones catalyzed by lipase B from *Candida antarctica*. The initiator efficiency in most of the cases was generally greater than 80%, and the percent of water-initiated chains was less than 30%. An efficient fractionation method was developed to remove homopolymer contamination from the block copolymers. Unlike published chemical methods to synthesize polybutadiene−polyester diblock copolymers, this enzymatic method did not require dry reaction conditions, exclusion of oxygen, or the use of highly reactive organometallic reagents.

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Supporting Information Available: ^1H NMR spectra of poly(BD-*b*-PDL), its oxalyl derivative, and poly(BD-*b*-CL). This material is available free of charge via the Internet at http://pubs.acs.org.

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