Ring-Opening Polymerization of *β*-Butyrolactone by Thermophilic Lipases

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Received May 23, 1997
Revised Manuscript Received July 31, 1997

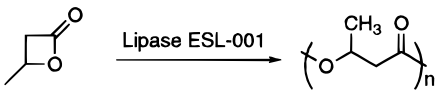
Introduction

Enzyme-catalyzed ring-opening polymerization of lactones has recently received much attention as a new approach for synthesis of polyesters, especially poly(hydroxyalkanoate)s (PHAs),[1,2] a family of biodegradable polymers which are of great interest for biomedical applications. Researchers including Gutman, Kobayashi, and Gross and their co-workers have shown that a number of lipases catalyze the ring-opening polymerization of ϵ-caprolactone affording polymers with molecular weights (M_n) from 1600 up to 7700 g/mol.[3−5] Mechanistic studies by Gross[6] suggested that porcine pancreatic lipase (PPL)-catalyzed polymerization of ϵ-caprolactone shares many features with immortal polymerization of ϵ-caprolactone initated by the aluminum porphyrin system.[7] Kobayashi and co-workers also reported the polymerization of δ-valerolactone and macrolactones 11-undecanolide, 12-dodecanolide, and 15-pentadenolide.[8−10] They found lipase-catalyzed polymerization of macrolides proceed much faster than that of ϵ-caprolactone.

So far there have been only limited studies on enzymatic ring-opening polymerization of smaller lactones. Matsumura *et al.* reported preparation of poly(β-D,L-malic acid) by lipase-catalyzed ring-opening polymerization of four-membered benzyl β-D,L-malolactonate.[11] Namekawa *et al.* examined ring-opening polymerization and copolymerization of β-propiolactone.[12] In a recent paper by Marchessault, polymers with 3−12 repeating units were obtained from lipase-catalyzed polymerization of β-butyrolactone.[13] The polymerization of γ-butyrolactone was also examined by the same authors. In addition, stereoselective ring-opening polymerization of α-methyl-β-propiolactone by lipase was just recently achieved by Gross and co-workers.[14]

"Thermophilic" enzymes, a novel family of enzymes cloned from exotic microorganisms in extreme environments, are currently receiving increasing research interests.[15−17] The commercially available thermophilic esterase/lipase (ESL-001) from Recombinant Biocatalysis Inc. (La Jolla, CA) currently consists of seven enzymes (called CloneZymes). The unique stability of these enzymes over a range of PH, temperature, and solvent conditions permits the exploration of reactions within much wider ranges of conditions without significant loss in enzymatic activity. Furthermore, they may also display unique regio-, chemo-, and stereoselectives. In this paper, we report an example of ring-opening polymerization of racemic β-butyrolactone catalyzed by thermophilic lipases from the ESL-001 ClonZyme library, where optically active(*R*-enriched poly(3-hydroxybutyrate) was produced (Scheme 1).

Scheme 1



Experimental Section

Materials. β-Butyrolactone was purchased from Aldrich Chemical Co. and used as received. THF (HPLC grade) was obtained from Fisher Scientific Inc. PPL (type II, approximately 25% protein) was purchased from Sigma Chemical Co. Lipase library CloneZyme ESL-001 was provided by Recombinant Biocatalysis Inc. (La Jolla, CA). The enzymes were lyophilized for 24 h before use for polymerization.

Polymerization. The general procedure is as follows: A mixture of β-butyrolactone (0.720 g) and lipase ESL-001 (8.4 mg) was stirred either in bulk or with isooctane (2 mL) as the solvent in a capped vial placed in a heated oil bath. After reaction, the resulted mixture was dissolved in chloroform and filtered and then washed with water to remove any enzyme (there exists a possibility that some amount of oligomers with very low molecular weight, if formed, might be removed during this treatment). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. Most unreacted monomers could be removed by repeated rotary evaporation with chloroform. The resulting substance was then placed under high vacuum overnight to remove volatiles including unreacted monomers. Water content in the system was determined by Karl Fischer titration to be 4.5 and 7.8 mg, respectively, for bulk polymerization and polymerization in isooctane.

Characterization and Analysis. The ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Varian VXR-400 spectrometer. For the polymer product: ^{1}H NMR ($CDCl_3$) δ 1.19 (s, CH_3), 2.36−2.53 (d, CH_2), 5.16 (s, CH) ppm; ^{13}C NMR ($CDCl_3$) δ 19.2 (CH_3), 40.3 (CH_2), 67.2 (CH), 168.8 (C═O) ppm. Molecular weights were determined by GPC using a Shodex GPC KF-806L column with a UV detector working at 254 nm. THF was used as the eluent at a flow rate of 1.0 mL/min. A calibration curve was generated with polystyrene standards with molecular weights of 7.6×10^2, 2.36×10^3, 3.7×10^3, 1.26×10^4, 4.4×10^4, 2.124×10^5, 9.35×10^5, and 1.88×10^6 (Aldrich).

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. To determine the stereoselectivity of the polymerization reaction, the resulted polymer was degraded by controlled methanolysis based on a literature method.[18,19] The methanolysis product was then analyzed by ^{1}H NMR using 10 mol % chiral shift reagent (+)-Eu(hfc)$_3$ in $CDCl_3$.

Results and Discussion

We first examined thermophilic lipase ESL-001-02 as a potential catalyst for the ring-opening polymerization of racemic β-butyrolactone. The reaction was performed either in bulk or in isooctane. Poly(3-hydroxybutyrate), PHB, was formed in good yield in both cases. Due to the unique thermal stability of the enzyme, the polymerization could be carried out at temperatures ranging from 60 to 80 °C. This greatly reduced the reaction time. During the polymerization no additional nucleophile was introduced. Trace water in the system acted as an initiator.

Polymerization results under different conditions are summarized in Table 1. It was found that polymerizations performed in bulk afforded higher molecular weights than those performed in isooctane. Also, higher monomer conversions were achieved in bulk. When performed in bulk at 60 °C with prolonged reaction time, the polymerization gave a polymer with a relatively lower molecular weight. This decrease in molecular weight was probably caused by chain degradation as suggested earlier by Gross.[6] Compared with Marchesault's results and our own experiments with PPL, the use of ESL-001-02 not only reduced the reaction time but also produced polymers with higher molecular weights.

The PHBs obtained from ESL-001-02 catalyzed ring-opening polymerization were found to be optically active.

S0024-9297(97)00722-5 CCC: $14.00 © 1997 American Chemical Society

Table 1. Lipase ESL-001-02 Catalyzed Ring-Opening Polymerization of β-Butyrolactone[a]

entry	temp, °C	solvent	time, h	yield, %	M_w[b]	M_w/M_n[b]	$[\alpha]_{365}^{25}$[c]	ee_p,[d] %
1	80	isooctane	66	51	2800	2.5	+5.3	37
2	80	bulk	66	62	3900	2.8	+4.1	27
3	70	isooctane	94	58	2900	2.9	+4.1	25
4	70	bulk	94	65	3800	2.9	+3.8	20
5	60	bulk	140	62	2300	2.7	+3.9	23

[a] The polymerization was carried out using 0.720 g of β-butyrolactone and 8.4 mg of the enzyme. [b] Determined by GPC. [c] In $CHCl_3$, c = 2.7. [d] Defined as $[(R - S)/(R + S)]$ for the polymer formed from the ring-opening polymerization.

Scheme 2

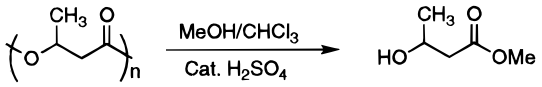


Table 2. Ring-Opening Polymerization of β-Butyrolactone Catalyzed by Thermophilic Lipase Library ESL-001[a]

enzyme	yield, %	M_w[b]	M_w/M_n[b]	$[\alpha]_{365}^{25}$[c]	ee_p,[d] %
ESL-001-01[e]	70	2700	2.4	+1.7	12
ESL-001-02	63	2400	2.3	+5.1	31
ESL-001-03	52	1200	2.2	+3.8	21
ESL-001-04	67	2600	2.4	+1.5	11
ESL-001-05	78	2100	2.7	+2.1	15
ESL-001-06	83	2700	3.0	+1.0	6
ESL-001-07	41	900	2.4	+5.6	40

[a] The polymerization was carried out in bulk at 75 °C for 108 h, using 0.720 g of β-butyrolactone and 8.4 mg of the enzyme. [b] Determined by GPC. [c] In $CHCl_3$, 2.7. [d] Defined as $[(R - S)/(R + S)]$ for the polymer formed from the ring-opening polymerization. [e] Polymerization using 500 mg of PPL under the same conditions afforded PHB in 85% yield with M_w = 1100, M_w/M_n = 2.3, [α] = +1.2, ee_p = 9%.

They showed the same sign of optical rotation with that of natural PHB which contains only (*R*)-hydroxybutyrate (HB) repeating units. This suggested that the polymers from the ring-opening polymerization were enriched with *R*-configuration repeating units. To evaluate the stereoselectivity, the resulting polymers were degraded by controlled methanolysis (Scheme 2). The *ee* values were then measured by ^{1}H NMR analysis of the methanolysis products using chiral shift reagent (+)-Eu(hfc)$_3$. As shown in Table 1, the polymerizations in isooctane resulted in higher selectivities than the bulk polymerizations under the same conditions. For example, polymerization carried out in isooctane at 80 °C afforded PHB with 37% *ee*, while the corresponding bulk polymerization produced an *ee* value of 27%.

According to the mechanism proposed for lipase-catalyzed polymerization of lactones, the polymerization was initiated by the reaction of enzyme with the lactone to form an acyl-enzyme intermediate. The intermediate then reacts either with a nucleophile to accomplish the initiation or with the hydroxyl group of a growing polymer chain to continue the propagation.[5,6,14] The formation of *R*-enriched polymers can be attributed to the rate difference between the reaction of the lipase with (*R*)- or (*S*)-β-butyrolactone and/or the rate difference between the reaction of the acyl-enzyme intermediate with *R*- or *S*-configuration chain ends. Further mechanistic studies are currently in progress.

Six other lipases from the thermophilic enzyme library Clonezyme ESL-001 were also examined. For comparison, the polymerizations were all conducted in bulk at 75 °C for 108 h. The results are presented in Table 2.

The ring-opening polymerization catalyzed by ESL-001-01, ESL-001-04 and ESL-001-06 gave the best results in terms of molecular weight. On the other hand, they demonstrated relatively low selectivity. The best stereoselectivity was obtained with ESL-001-07 and ESL-001-02, with *ee* values of 40% and 31%, respectively. The polymer from ESL-001-07 catalyzed polymerization had a low molecular weight (M_w) of only 900 g/mol. As seen in Table 2, ESL-001-02 turned out to be the best candidate catalyst from the viewpoint of both molecular weight and stereoselectivity.

In summary, this thermophilic lipase-catalyzed stereoselective ring-opening polymerization of racemic β-butyrolactone presents an unique approach to the synthesis of optically active PHB enriched with *R*-repeating units.

Acknowledgment. This work was supported by Hercules Inc. and by a research grant (449-HF97) in agricultural chemistry from Herman Frasch Foundation. We also thank Recombinant Biocatalysis Inc. for providing the thermophilic lipases.

Supporting Information Available: Figures containing ^{1}H and ^{13}C NMR of β-butyrolactone and the polymerization product and ^{1}H NMR of the reaction mixture from the control experiment without enzyme, the reaction mixture from enzyme-catalyzed polymerization, and methyl 3-hydroxybutyrate (7 pages). Ordering information is given on any current masthead page.

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MA970722J