Lipase-Catalyzed Ring-Opening Polymerization of β-Butyrolactone to the Cyclic and Linear Poly(3-hydroxybutyrate)

Shuichi Matsumura,* Yoichi Suzuki, Keisuke Tsukada, and Kazunobu Toshima

Faculty of Science and Technology, Keio University, 3-14-1, Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan

Yoshiharu Doi and Ken-ichi Kasuya

Polymer Chemistry Laboratory, The Institute of Physical and Chemical Research (RIKEN), 2-1, Hirosawa, Wako-shi, Saitama 351-0198, Japan

Received December 30, 1997; Revised Manuscript Received July 23, 1998

ABSTRACT: The four-membered β-butyrolactone (BL) was polymerized using a ring-opening polymerization reaction with lipase as a catalyst at a temperature between 60 and 100 °C to yield the corresponding cyclic and linear poly(3-hydroxybutyrate)s [P(3HB)] with weight-average molecular weights of up to 7300. Among the tested lipases, porcine pancreatic lipase (PPL) and *Candida cylindracea* lipase (CC) showed the best results with respect to the molecular weight of the resulting polymer and the monomer conversion. It was found that a significant amount of the cyclic P(3HB) fraction in the resulting polymer was produced and the cyclic polymer was increased with increasing monomer conversion. The (*R*)-BL was more easily polymerized by lipase to form the corresponding P(3HB) having a higher molecular weight than that formed by using (*R,S*)-BL.

Introduction

Microbially produced poly[(*R*)-3-hydroxybutyrate] [(*R*)-P(3HB)] and its copolymers containing (*R*)-3-hydroxyalkanoate monomeric units are now being extensively studied, and their applications as biodegradable and biocompatible thermoplastic materials in both the industrial and medicinal fields are rapidly being advanced.[1] The molecular weights of microbially produced P(3HB) often exceed approximately 10^6. On the other hand, relatively low-molecular weight P(3HB)s of a few thousands have attracted attention as novel biodegradable and biocompatible polymers,[2] which may have applications as plasticizers for high molecular weight materials, for drug delivery systems,[3] or for the synthetic blocks for copolymers.[4] They can be prepared by degradation methods such as pyrolysis,[5] partial depolymerization,[6] or partial transesterification. However, molecular weight control will be rather difficult, and possible molecular structures are dependent on the parent structures of P(3HB) and its copolymers. Apart from the degradation method, there may be a polymerization of the corresponding monomers.[7] However, conventional polymerizations of β-butyrolactone (BL) require extremely pure monomers and anhydrous conditions as well as metallic catalysts which must be completely removed before use, particularly for medical applications. To avoid these difficult restrictions for the polymerizations of BL by chemical methods, enzymatic polymerization may be one of the more feasible methods to obtain the polyesters. Enzyme-catalyzed polymerization of β-lactones should offer a feasible way to design and synthesis of the relatively low molecular weight P(3HB) and its copolymers, because lipases can accept a wide range of substrates, including cyclic lactones, to produce a wide range of polyesters. Furthermore, lipase-catalyzed polymerization may be one of the most attractive applications in the industrial field for the next generation, because enzymatically polymerized polyesters are expected to be biodegradable.

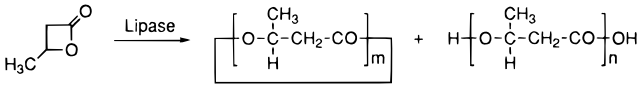
The lipase-catalyzed ring-opening polymerization of the four-membered BL was first reported by Nobes et al.[8] P(3HB) having weight-average molecular weights ($\bar{M}_w$) ranging from 256 to 1045 were prepared after several weeks of polymerization using approximately equal weights of BL and lipase. Very recently, a much improved enzyme-catalyzed polymerization of BL was reported using thermophilic lipases to yield optically active P(3HB) enriched with *R*-repeating units having an $\bar{M}_w$ ranging from 900 to 3900.[9] In addition to BL, the lipase-catalyzed ring-opening polymerization of four-membered lactones, benzyl β-malolactonate,[10] α-methyl-β-propiolactone,[11] and β-propiolactone[12,13] was reported. However, details of the enzymatically polymerized P(3HB), such as enantioselectivity, end groups of the polymer chain, and linear or cyclic structures, were not extensively studied. In this paper, the lipase-catalyzed polymerization of BL to P(3HB) having an $\bar{M}_w$ of up to 7300, the preferential polymerization of the (*R)*-BL by lipase, and the resulting P(3HB) containing a significant amount of cyclic polymer were reported.

Experimental Section

Materials. (*R,S*)-β-Butyrolactone (BL) was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), and used after distillation in a reduced pressure. (*R*)-Enriched BL was obtained by the enzymatic resolution of (*R,S*)-BL using porcine pancreatic lipase (PPL) basically according to the literature.[14] (*R*)-Enriched BL was purified by silica gel column chromatography using chloroform. The prepared (*R*)-enriched BL having 41% ee and 92% ee were used in this study. Enzymes: Porcine pancreatic lipase (PPL, 190 unit/mg protein, using olive oil, according to the supplier), purified porcine pancreatic lipase (30000 unit/mg protein, using olive oil, according to the supplier) and lipase from *Candida cylindracea* (CC, 943 unit/mg solid, using olive oil, according to the supplier) were purchased from Sigma Chemical Co. (St. Louis, MO). Lipase PS was kindly supplied by Amano Pharmaceutical Co., Ltd. (Nagoya, Japan). Novozym 435 (triacylglycerol hydrolase + carboxylesterase) having 7000 PLU/g (propyl laurate units)

S0024-9297(97)01894-9 CCC: $15.00
Published on Web 09/04/1998

Scheme 1



and Lipozyme IM were kindly supplied by Novo Nordisk A/S (Bagsvaerd, Denmark). Lipase M was kindly supplied by Amano Pharmaceutical Co., Ltd. (Nagoya, Japan). The enzymes were used without further purification.

Polymerization. The enzymatic ring-opening polymerization of BL was carried out as shown in Scheme 1. The general procedures are as follows. A mixture of BL (200 mg) and enzyme was stirred in bulk under an argon atmosphere in a capped vial placed in a thermostated oil bath. After the reaction, the reaction mixture was dissolved in chloroform (10 mL), and the insoluble enzyme was removed by filtration. The chloroform was then evaporated under slight reduced pressure to quantitatively obtain the polymer mixture. The molecular weight and the molecular weight dispersion of the polymer fraction were measured by size exclusion chromatography (SEC). The monomer conversion of BL to P(3HB) was determined by comparison of the ^{1}H NMR spectral integration intensities for the $\delta = 1.58$ ppm peak corresponding to the methyl protons of monomeric BL with the corresponding methyl protons of P(3HB) at $\delta = 1.26$ ppm. The polymer structure was analyzed by ^{1}H and ^{13}C NMR, IR, elemental analysis and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI−TOF MS).[15]

Measurements. The weight-average molecular weight ($\bar{M}_w$), number-average molecular weight ($\bar{M}_n$) and molecular weight dispersion ($\bar{M}_w/\bar{M}_n$) were measured by a size exclusion chromatography (SEC) using SEC columns (Shodex K-803L + K-8006 + K-800D, Showa Denko Co., Ltd., Tokyo, Japan) with a refractive index detector. Chloroform was used as the eluent. The SEC system was calibrated with polystyrene standards of narrow molecular weight distribution. ^{1}H NMR spectra were recorded with a JEOL Model GSX-270 (270 MHz) spectrometer (JEOL Ltd., Tokyo, Japan). ^{13}C NMR spectra were recorded with a JEOL model JNM-FX90A Fourier transform spectrometer operating at 22.5 MHz with complete proton decoupling. Infrared (IR) spectra were measured using a JASCO Fourier transform spectrometer model FT/IR-5000 (JASCO Ltd., Tokyo, Japan). The matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI−TOF MS) was measured with a Bruker Proflex mass spectrometer. The spectrometer was equipped with a nitrogen laser. The detection was in the reflector and linear mode. 2,5-Dihydroxybenzoic acid (DHBA) was used as the matrix, and positive ionization was used.

Results and Discussion

It was found that BL was polymerized in bulk by lipase to yield P(3HB) with a good monomer conversion. Table 1 shows the typical ring-opening polymerization of BL with and without enzyme. It was found that the polymerization of BL could be carried out at temperatures ranging from 60 to 100 °C, and the highest $\bar{M}_w$ were produced in bulk at 80 and 100 °C for *(R,S)*-BL and *(R)*-BL, respectively. BL was thermally oligomerized at 100 °C (Table 1, entry 11). However, both the $\bar{M}_w$ and the monomer conversion were significantly lower than was found when the reaction was carried out in the presence of PPL (Table 1, entries 4−9). These results indicate that the lipase actually promoted the polymerization. Purified porcine pancreatic lipase having 30 000 units/mg of protein was used in order to compare the polymerization results using crude PPL powder having 190 unit/mg protein. It was confirmed that the similar polymerization of BL occurred using both the purified porcine pancreatic lipase and the crude PPL powder. This indicates that the PPL enzyme actually catalyzed the polymerization of BL, and no significant effects due to the impurities of the crude PPL powder were observed. So, in this report, crude PPL powder was used in further studies. It was observed that (*R,S*)-BL was polymerized with all lipases tested at 80 °C for 5 d. Among the enzymes tested, PPL and CC showed the best results with respect to $\bar{M}_w$ and monomer conversion (Table 1, entries 12−20). Figure 1 shows the $\bar{M}_w$ and the monomer conversion of (*R,S*)-BL as a function of lipase concentration at 80 and 100 °C using CC after a 5 d polymerization of (*R,S*)-BL. It was found that the $\bar{M}_w$ of the resulting P(3HB) was significantly influenced by the lipase concentration. The highest $\bar{M}_w$ was obtained at a lipase concentration of 5% after a 5 d polymerization of (*R,S*)-BL. Though the monomer conversion was higher at 100 °C compared to that at 80 °C, the $\bar{M}_w$ of the resulting P(3HB) was higher at 80 °C than that at 100 °C after a 5 d polymerization when compared on the same lipase concentration basis. It was found that a 5% CC concentration seemed to be the best for the polymerization of (*R,S*)-BL using CC with respect to the $\bar{M}_w$ and the monomer conversion. This tendency was in agreement with the lipase-catalyzed ring-opening polymerization of β-propiolactone.[12] Figure 2 shows the time course of the polymerization of *(R,S)*-BL using 5% CC at 80 and 100 °C. At 100 °C, the $\bar{M}_w$ quickly increased to the maximum value within the first 1 d and remained almost constant during additional polymerization. However, the monomer conversion was gradually increased after the $\bar{M}_w$ reached the maximum value. This is probably ascribed to the transesterification or chain degradation reaction that occurred at the higher temperature of 100 °C. It was found that the rate of monomer conversion and increase in $\bar{M}_w$ were faster by increasing the polymerization temperature from 80 to 100 °C. On the other hand, the $\bar{M}_w$ after a 5-day polymerization was higher at 80 °C.

To evaluate the enantioselectivity of the lipase-catalyzed ring-opening polymerization of (*R,S*)-BL, (*R,S*)-BL was polymerized to about 50% monomer conversion using 5% PPL at 80 °C and the specific optical rotation of the residual BL monomer was measured. The unreacted monomeric BL was recovered by silica gel column chromatography from the polymerization mixture. It was found that the specific optical rotation of the BL monomer was $[\alpha]^{31}_{589} = -1.03°$ ($c = 1$). When compared to the [α] value of pure *(S)*-BL being $[\alpha]^{25}_{589} = -26.1°$ ($c = 5$),[16] it is indicated that a slight *R*-enantioselection occurred during the lipase-catalyzed ring-opening polymerization of *(R,S)*-BL using PPL. These results of *R*-selection were in agreement with those obtained using thermophilic lipases by Xie et al.[9] According to the mechanism proposed for the lipase-catalyzed polymerization of lactones, the polymerization was initiated by the reaction of the enzyme with the lactone to form an acyl−enzyme complex, or enzyme-activated monomer (EAM). The EAM then reacts either with a nucleophile, such as water, which is perhaps contained in the enzyme, to accomplish the initiation or with the hydroxyl group of a growing polymer chain to continue the propagation.[9,17−20] However, the enzyme-catalyzed polymerization of BL may be affected by the polymer (oligomer) structures that EAM attacks. In other words, isotactic and syndiotactic structures of P(3HB) may vary the reactivity with the EAM. Therefore, *(R,S)*-BL and

Table 1. Typical Ring-Opening Polymerization of β-Butyrolactone (BL) with/without Enzyme

entry[a]	BL[b]	enzyme[c]	wt %	temp (°c)	time (h)	conv (%)	$\bar{M}_w$[d]	$\bar{M}_w/\bar{M}_n$
1	*R*	PPL	5	40	48	4	370	1.4
2	*R*	PPL	5	60	48	83	400	1.5
3	*R*	PPL	5	80	48	92	4000	1.3
4	*R*	PPL	5	100	48	99	5600	2.0
5	*R*	PPL	0.5	100	72	34	2800	2.3
6	*R*	PPL	1	100	72	83	5700	2.8
7	*R*	PPL	3	100	24	99	7300	1.9
8	*R*	PPL	5	100	24	99	5600	1.8
9	*R*	PPL	10	100	12	95	3600	1.7
10	*R*		0	80	48	0		
11	*R*		0	100	48	4	260	
12	*R,S*	PPL	1	80	120	56	2200	2.2
13	*R,S*	PPL	5	80	120	70	3100	1.3
14	*R,S*	CC	1	80	120	31	1600	1.7
15	*R,S*	CC	5	80	120	73	3300	1.3
16	*R,S*	PS	1	80	120	16	1400	1.9
17	*R,S*	M	1	80	120	38	1500	2.0
18	*R,S*	Lipo	1	80	120	18	3400	3.0
19	*R,S*	Novo	1	80	120	7	1000	1.4
20	*R,S*		0	80	120	6		
21	*R,S*	PPL	5	100	120	94	3100	1.3
22	*R,S*	CC	5	100	120	93	2800	1.6
23	*R,S*	PS	5	100	120	95	1900	1.8
24	*R,S*	M	5	100	120	97	2600	1.7
25	*R,S*	Lipo	5	100	120	95	2800	2.1
26	*R,S*	Novo	5	100	120	74	2000	2.0
27	*R,S*		0	100	120	24	1000	1.5

[a] Entries 10, 11, 20, and 27 were blank tests. [b] (*R*)-BL: 92% ee (*R*)-BL. [c] Key: PPL, porcine pancreatic lipase; CC, *C. cylindracea* lipase; PS, lipase PS; M, lipase M; Novo, Novozym 435; Lipo, Lipozyme IM. [d] Determined by SEC analysis, calibrated with polystyrene standards.

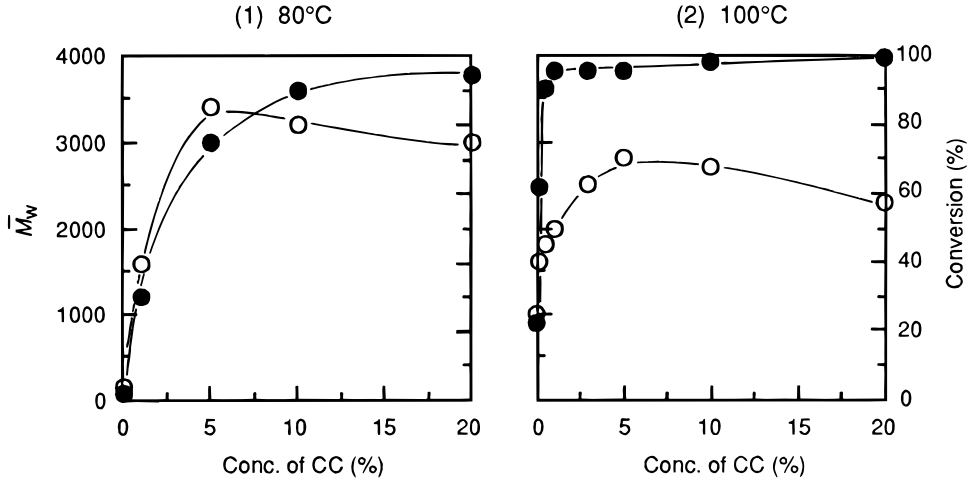


Figure 1. Lipase-catalyzed polymerization of (*R,S*)-BL using CC for 5 d at 80 and 100 °C in bulk. Key: (○) $\bar{M}_w$; (●) monomer conversion.

(R)-BL were separately polymerized and their polymerization results compared. Figure 3 shows the relationship between molecular weight and monomer conversion as a function of the polymerization time of BL having varying degrees of *R*-enantiomeric excess using 5% PPL at 100 °C in bulk. It was found that BL was quickly polymerized to a maximum $\bar{M}_w$ after about 24 h and then the $\bar{M}_w$ remained constant. The $\bar{M}_w$ of P(3HB) was higher by increasing the *R*-enantiomeric excess of the monomeric BL. Similar tendencies were observed for the monomer conversion as shown in Figure 3. That is, (*R*)-BL with 92% ee was polymerized with 99% monomer conversion after 24 h; however, (*R,S*)-BL was polymerized with 75% conversion after 24 h and 89% monomer conversion after 72 h polymerization. From these results, it was indicated that the P(3HB) consisting of (*R*)-BL units was more easily polymerized by the reaction of EAM and reached a higher M_w than the corresponding (*R,S*)-P(3HB).

A typical MALDI−TOF mass spectrum, recorded in reflector mode, of (*R*)-P(3HB) obtained with a medium conversion (71%) during bulk polymerization of (*R*)-BL with 92% ee using 5% PPL at 100 °C after 5 h is shown in Figure 4. The ionization process of a neutral macromolecule in MALDI−TOF proceeds through the capture of a proton or a metal ion, usually sodium, which forms a charged adduct with the molecular species.[21] MALDI−TOF mass spectroscopy showed that P(3HB) produced by the ring-opening polymerization of *(R)*-BL had two structural isomers consisting of a linear P(3HB) and cyclic P(3HB) as shown in Scheme 1. The mass differences between each of the two peaks were 18 *m/z*. Figure 4 also shows an expanded portion of the spectrum with two distinct mass series. One is due to the

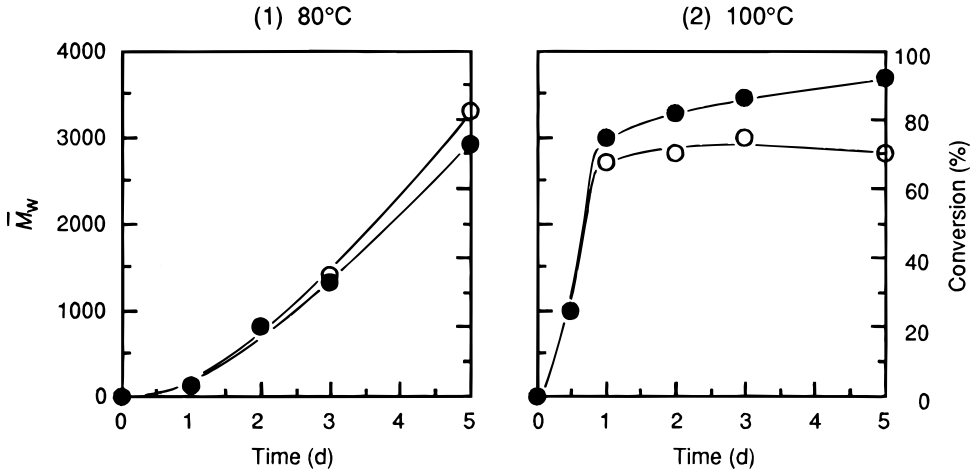


Figure 2. Time-course of lipase-catalyzed polymerization of (*R*,*S*)-BL using 5% CC at 80 and 100 °C in bulk. Key: (○) $\bar{M}_w$; (●) monomer conversion.

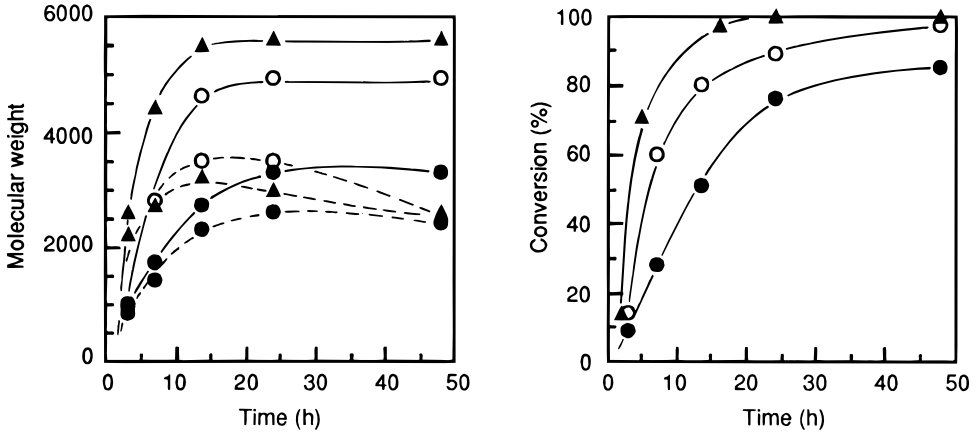


Figure 3. Time-course of lipase-catalyzed polymerization of BL using 5% PPL at 100 °C in bulk. Key: (▲) *R* (92% ee), (○) *R* (41% ee); (●) *R*,*S*; (—) $\bar{M}_w$; (- - -) M_n.

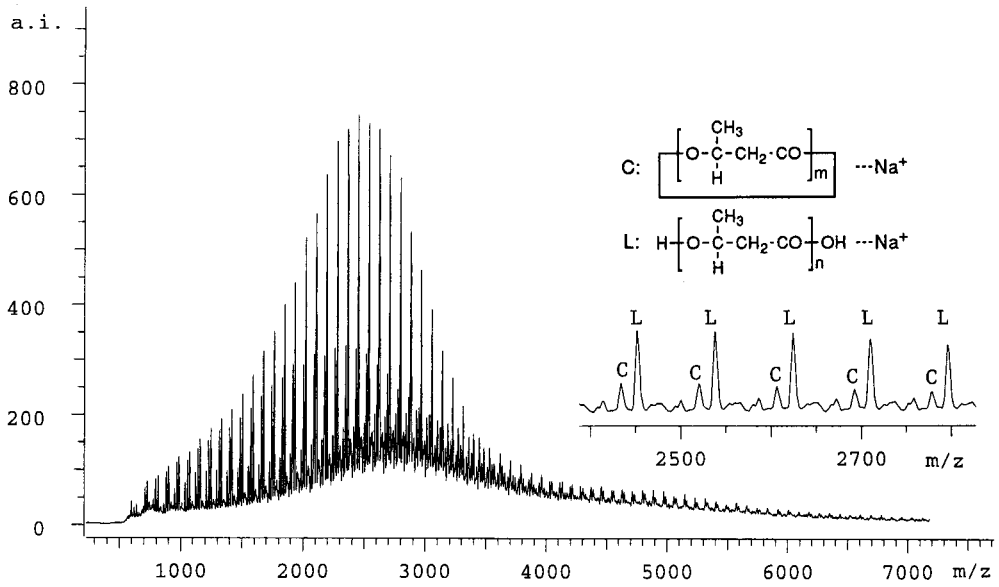


Figure 4. MALDI−TOF mass spectrum of (*R*)-P(3HB), recorded in reflector mode using DHBA as matrix, with a medium conversion (71%). Bulk polymerization of (*R*)-BL with 92% ee using 5% PPL at 100 °C for 5 h.

linear P(3HB) clustered to Na^+ ions and terminated by carboxyl and hydroxyl end groups. The other is due to the cyclic P(3HB) clustered to Na^+ ions. The cyclic P(3HB) contained approximately 30 mol % in the P(3HB) obtained during the medium conversion. To confirm the linear and cyclic structures of P(3HB) in the MALDI−TOF mass spectrum, trifluoroacetic anhydride was added to the polymer sample. By the addition of trifluoroacetic anhydride, the hydroxyl group of the linear P(3HB) reacted to yield the corresponding tri-

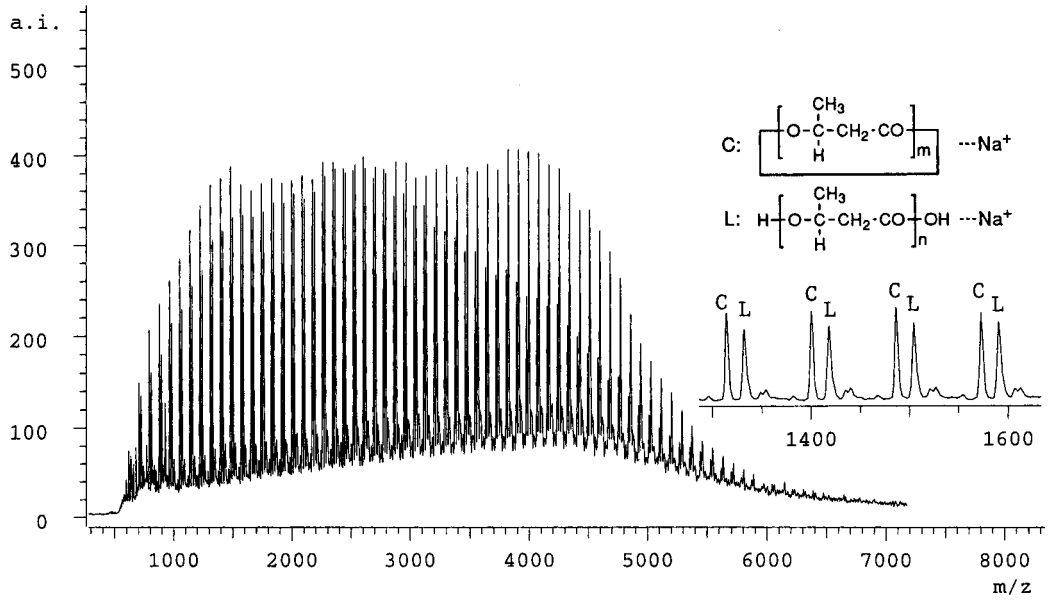


Figure 5. MALDI−TOF mass spectrum of (*R*)-P(3HB), recorded in reflector mode using DHBA as matrix, with a high conversion (99%). Bulk polymerization of (*R*)-BL with 92% ee using 5% PPL at 100 °C for 24 h.

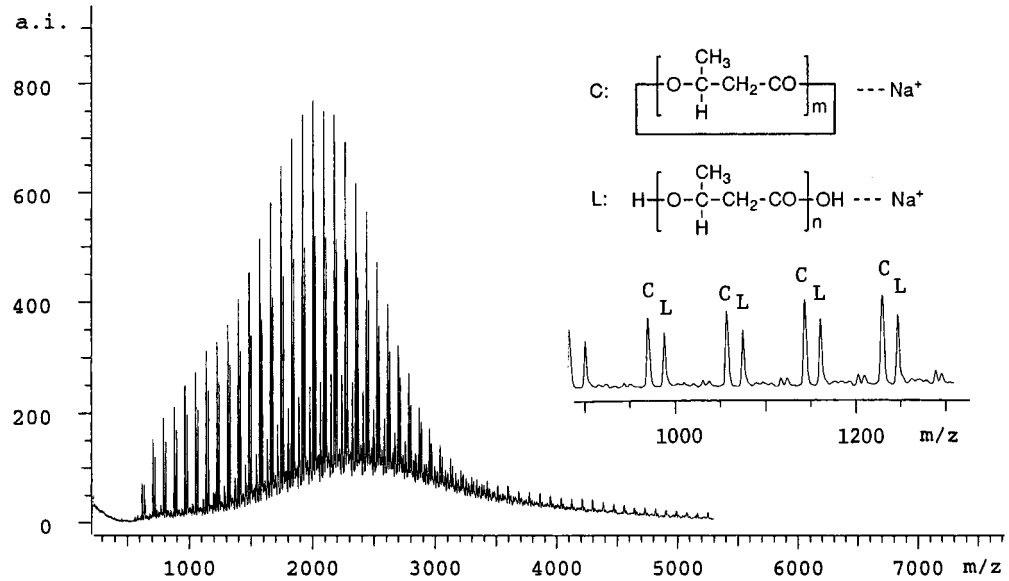


Figure 6. MALDI−TOF mass spectrum of (*R,S*)-P(3HB), recorded in reflector mode using DHBA as matrix. Bulk polymerization of (*R,S*)-BL using 5% PPL at 80 °C for 48 h.

fluoroacetate, which produced a peak shift of +96 *m/z* for each peak of the linear P(3HB). On the other hand, peaks corresponding to the cyclic P(3HB) from the MALDI−TOF mass spectroscopy remained unchanged.

Figure 5 shows the MALDI−TOF mass spectrum, recorded in reflector mode, of (*R*)-P(3HB) obtained in a high conversion (99%) during bulk polymerization of (*R*)-BL with 92% ee using 5% PPL at 100 °C after 24 h. It was observed that the molecular weight dispersion increased with increasing polymerization time. Figure 5 also shows an expanded portion of the spectrum with two distinct mass species due to the linear and cyclic P(3HB)s clustered to Na^+ ions. It was found that P(3HB) after the higher conversion contained a considerable amount of cyclic P(3HB) compared to linear P(3HB). Cyclic P(3HB) was the major component in the lower mass region of less than 2600 with a $\bar{M}_w$ distribution centered around 2100, whereas the linear P(3HB) appeared more abundantly at the higher mass of greater than 2600 with a $\bar{M}_w$ distribution centered around 3200. This broad molecular weight dispersion might be ascribed to the transesterification reaction by the prolonged reaction at 100 °C. Similar results were obtained using *(R,S)*-BL with lipases as shown in Table 1. However, there is no clear relations of the cyclic and linear P(3HB) ratios in the lipase-catalyzed polymerization of BL. Figure 6 shows the MALDI−TOF mass spectrum, recorded in reflector mode, of P(3HB) obtained by the bulk polymerization of *(R,S)*-BL using 5% PPL at 80 °C for 48 h as a typical example. It was confirmed that cyclic P(3HB) was produced as the major components. Further studies are now under way.

Conclusions

In conclusion, it was found that a four-membered BL was polymerized by lipase as a catalyst to yield the

corresponding cyclic and linear P(3HB) with an $\bar{M}_w$ of up to 7300. The amount of cyclic P(3HB) was increased by increasing the monomer conversion. Among the lipases tested, PPL and CC showed the best results with respect to both the polymerization rate and the molecular weight of the resulting P(3HB). The (*R*)-BL was more quickly polymerized compared to (*R*,*S*)-BL to produce the corresponding P(3HB).

Acknowledgment. This work was supported by a research grant of Keio University Special Grant-in-Aid for Innovative Collaborative Research Projects. We acknowledge the gift of lipases from Novo Nordisk A/S and Amano Pharmaceutical Co., Ltd.

References and Notes

(1) (a) Dawes, E. A.; Senior, P. J. *Adv. Microb. Physiol.* **1973**, *10*, 135. (b) Doi, Y. *Microbial Polyesters*; VCH Publishers: New York, 1990. (c) Steinbüchel, A.; Valentin, H. E. *FEMS Microbiol. Lett.* **1995**, *128*, 219.

(2) Bürger, H. M.; Müller, H.-M.; Seebach, D.; Börnsen, K. O.; Schär, M.; Widmer, H. M. *Macromolecules* **1993**, *26*, 4783.

(3) Lafferty, R. M.; Korsatko, B.; Korsatko, W. In *Biotechnology*; Rehm, H. J., Ed.; Verlag Chemie: Weinheim, Germany, 1988; Vol. 6b, p 135.

(4) Reeve, M. S.; McCarthy, S.; Gross, R. A. *Polym. Prepr.* **1990**, *31* (1), 437.

(5) (a) Morikawa, H.; Marchessault, R. H. *Can. J. Chem.* **1981**, *59*, 2306. (b) Kunioka, M.; Doi, Y. *Macromolecules* **1990**, *23*, 1933.

(6) Seebach, D.; Beck, A. K.; Brandli, U.; Muller, D.; Przybylski, M.; Schneider, K. *Chimia* **1990**, *44*, 112.

(7) (a) Hori, Y.; Suzuki, M.; Yamaguchi, A.; Nishishita, T. *Macromolecules* **1993**, *26*, 5533. (b) Hori, Y.; Takahashi, Y.; Yamaguchi, A.; Nishishita, T. *Macromolecules* **1993**, *26*, 4388. (c) Abe, H.; Matsubara, I.; Doi, Y.; Yamaguchi, A. *Macromolecules* **1994**, *27*, 6018.

(8) Nobes, G. A. R.; Kazlauskas, R. J.; Marchessault, R. H. *Macromolecules* **1996**, *29*, 4829.

(9) Xie, W.; Li, J.; Chen, D.; Wang, P. G. *Macromolecules* **1997**, *30*, 6997.

(10) Matsumura, S.; Beppu, H.; Nakamura, K.; Osanai, S.; Toshima, K. *Chem. Lett.* **1996**, 795.

(11) Svirkin, Y. Y.; Xu, J.; Gross, R. A.; Kaplan, D. L.; Swift, G. *Macromolecules*, **1996**, *29*, 4591.

(12) Matsumura, S.; Beppu, H.; Tsukada, K.; Toshima, K. *Biotechnol. Lett.* **1996**, *18*, 1041.

(13) Namekawa, S.; Uyama, H.; Kobayashi, S. *Polym. J.* **1996**, *28*, 730.

(14) Xu, J.; Gross, R. A.; Kaplan, D. L.; Swift, G. *Macromolecules* **1996**, *29*, 3857.

(15) The spectral data and elemental analysis of (*R*)-P(3HB) having an $\bar{M}_w$ of 5600 are shown (entry 4 in Table 1) to be representative. IR (KBr): 2984 (CH_2), 1748, 1184 (ester C=O) cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ = 1.3 (d, 3H, 5.0), 2.6 (m, 2H), 5.3 (m, 1H, m). ^{13}C NMR (22.5 MHz, $CDCl_3$): δ = 19.9 (CH_3), 40.9 (CH2), 67.7 (CH), 169.3 (ester C=O). Anal. Calcd for $(C_4H_6O_2)_n$: C, 55.81; H, 7.02. Found: C, 55.94; H, 7.40.

(16) Zang, Y.; Gross, R. A.; Lenz, R. W. *Macromolecules* **1990**, *23*, 3206.

(17) MacDonald, R. T.; Pulapura, S. K.; Svirkin, Y. Y.; Gross. R. A.; Kaplan, D. L.; Swift, G.; Wolk, S. *Macromolecules* **1995**, *28*, 73.

(18) Henderson, L. A.; Svirkin, Y. Y.; Gross, R. A.; Kaplan, D.; Swift, G. *Macromolecules* **1996**, *29*, 7759.

(19) Svirkin, Y. Y.; Xu, J.; Gross, R. A.; Kaplan, L.; Swift, G. *Macromolecules* **1996**, *29*, 4591.

(20) Kobayashi, S.; Uyama, H. In *Enzymes in Polymer Synthesis;* Gross, R. A., Kaplan, D. L., Swift, G., Eds.; ACS Symposium Series 684; American Chemical Society: Washington, DC, 1998; p 58.

(21) Montaudo, G.; Montaudo, M. S.; Puglisi, C.; Samperi, F.; Spassky, N.; LeBorgne, A.; Wisniewski, M. *Macromolecules* **1996**, *29*, 6461.

MA971894J