Lipase-catalyzed ring-opening polymerization of 16-hexadecanolide

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(Communicated by Seizo OKAMURA, M.J.A., April 13, 1998)

Abstract: Enzymatic ring-opening polymerization of a 17-membered lactone, 16-hexadecanolide (HDL), was performed in bulk by using lipases of different origin as catalyst. *Pseudomonas fluorescens* lipase exhibited high catalytic activity toward the polymerization, producing the corresponding polymer in high yields. A higher polymerization temperature (75°C) resulted in the formation of the polymer with molecular weight of more than 5×10^3 . HDL monomer was recovered unchanged in the polymerization without the enzyme. Michaelis-Menten kinetics of lactones in five different ring size showed that HDL had the largest enzymatic polymerizability among the lactones examined.

Key words: Enzymatic polymerization; 16-hexadecanolide; lipase; lactone; polyester.

Introduction. Recently, polyester synthesis by various monomer combinations through lipase catalysis has been extensively investigated.^{1)–4)} In case of the enzymatic polycondensations, activated diesters having 2,2,2-trifluoroethyl, 2,2,2-trichloroethyl, or vinyl leaving group have been often used as monomer to produce polyesters with high molecular weight under mild conditions. Furthermore, an optical active polyester was enantioselectively synthesized by lipase-catalyzed polycondensation of a racemic epoxide-containing activated diester with a diol monomer.⁵⁾

Lipase also induced ring-opening polymerization and copolymerization of lactones,^{6)–18)} lactide,^{14),19)} and six-membered cyclic carbonate.^{20)–22)} Until now, small-size (4-membered)^{6)–10)} and medium-size lactones^{11)–14)} (6- and 7-membered) as well as macrolides (12-, 13-, and 16-membered)^{15)–18)} were subjected to the lipase-catalyzed polymerization. Normally, macrolides show much lower reactivity and polymerizability in an anionic process than ϵ -caprolactone (ϵ -CL) due to their lower ring strain.⁴⁾ However, they were enzymatically polymerized much faster than ϵ -CL.^{15)–17)} This is probably due to a favored transition state of the macrolide to open the ring with lipase catalysis.²³⁾

In this study, a 17-membered lactone, 16-hexadecanolide (HDL), was used as a new monomer of lipase-

catalyzed ring-opening polymerization. Polymerization of HDL had been investigated by neither chemical nor enzymatic catalysts.

Results and discussion. The lipase-catalyzed ring-opening polymerization of HDL was carried out in bulk at 60°C for 5 days. In this study, commercially available five lipases were used as catalyst: lipases derived from *Candida antarctica* (lipase CA), *Candida cylindracea* (lipase CC), *Pseudomonas cepacia* (lipase PC), *Pseudomonas fluorescens* (lipase PF), and porcine pancreas (PPL). We reported that these enzymes could induce the ring-opening polymerization of lactones in various ring-sizes. Lipase CA is an immobilized enzyme on polymer beads and others are powdery enzymes. Polymerization results are summarized in Table I.

Among the enzymes examined, lipase PF showed the highest catalytic activity for the polymerization of HDL; the monomer was quantitatively consumed (entry 7). In case of lipase PC, the monomer conversion was relatively high (entry 4), whereas lipases CA, CC, and PPL had less catalytic activity (entries 1, 2, and 9). The molecular weight was determined by size exclusion chromatography (SEC). The molecular weight of the polymer obtained at 60°C was ca. 2000. HDL monomer was recovered unreacted in the polymerization without the enzyme (control experiment) (entry 10). These data indicate that the present polymerization proceeded via enzyme catalysis and the enzyme origin affected the polymerization behavior. The polymer structure was confirmed by ¹H NMR spectroscopy.

Effect of the polymerization temperature was ex-

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aminated by using lipases PC and PF (entries 3–8). In using lipase PC catalyst, the monomer conversion at 45°C was much lower than that at 60 or 75°C (entries 3–5). Lipase PF showed the high catalytic activity in the range from 45°C to 75°C (entries 6–8). The molecular weight increased as a function of the temperature. The highest molecular weight (5800) was achieved by the polymerization catalyzed by lipase PC at 75°C (entry 5).

The polymerization mechanism is first proposed as follows.¹⁵⁾ At first, the lactone is recognized and subjected to the ring-opening in the active center of lipase (serine residue) to give the acyl-enzyme intermediate. In the initiation step, water attacks nucleophilically to the intermediate, leading to the formation of the oxyacid, the shortest propagating species. The propagation step is the reaction of the intermediate with the terminal hydroxyl group of the polymer to produce the one-unit more elongated polymer chain.

Our previous studies show that 12-, 13-, and 16-membered macrolides having much lower anionic polymerizability than ϵ -CL exhibited unusually high reactivity through lipase catalysis;^{15)–17)} the lipase-catalyzed polymerization of the macrolides proceeded much faster than that of ϵ -CL, which is the reversed tendency of chemical reactions such as an alkaline hydrolysis and anionic ring-opening polymerizations.⁴⁾ Very recently, we carried out Michaelis-Menten kinetics of the polymerization of 7- and

13-membered lactones in order to quantitatively evaluate the lipase-catalyzed polymerizability.²³⁾ The kinetic study shows that the rate-determining step is the formation of the intermediate, therefore, the polymerization proceeds via a “monomer-activated mechanism”.

In this study, Michaelis-Menten kinetic parameters of HDL as well as 12- and 16-membered lactones were determined. The polymerization was carried out by using lipase PF catalyst in the presence of 1-octanol. For all monomers, linearity was observed in the Hanes-Woolf plot, indicating that the polymerization followed Michaelis-Menten kinetics. The parameters obtained are summarized in Table II. $V_{\max(\text{lactone})}/K_{\text{m}(\text{lactone})}$ of HDL was the largest, indicating that HDL had the largest enzymatic polymerizability among the lactones examined.

The larger the ring size of lactone, the larger the $V_{\max(\text{lactone})}/K_{\text{m}(\text{lactone})}$ value. $K_{\text{m}(\text{lactone})}$ values were not so different with each other, on the other hand, $V_{\max(\text{lactone})}$ increased with increasing the ring size. These data imply

Scheme 1

Table I. Lipase-catalyzed ring-opening polymerization of HDL^{a)}

Entry	Enzyme	Temp. (°C)	Conv. (%) ^{b)}	Mn ^{b)}	Mw/Mn ^{b)}
1	Lipase CA	60	37	2900	2.9
2	Lipase CC	60	12	2800	1.3
3	Lipase PC	45	11	1400	1.1
4	Lipase PC	60	78	2000	2.8
5	Lipase PC	75	100	5800	2.0
6	Lipase PF	45	85	2100	5.4
7	Lipase PF	60	99	2600	3.8
8	Lipase PF	75	97	5500	2.0
9	PPL	60	36	1700	1.3
10	— ^{c)}	60	0		

a) Polymerization of HDL (1 mmol) using lipase catalyst (50 mg) in bulk for 5 days. b) Determined by SEC using chloroform eluent. c) Without enzyme.

Scheme 2

Table II. Michaelis-Menten kinetic parameters in ring-opening polymerization of lactones catalyzed by lipase PF^{a)}

Ring Size of Lactone	$V_{\max(\text{lactone})}$ ($\times 10^2, \text{mol} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$)	$K_{\text{m}(\text{lactone})}$ ($\text{mol} \cdot \text{L}^{-1}$)	$V_{\max(\text{lactone})} /$ $K_{\text{m}(\text{lactone})}$ ($\times 10^2, \text{h}^{-1}$)
7 ^{b)}	0.66	0.61	1.1
12 ^{c)}	0.78	0.58	1.4
13 ^{b)}	2.3	1.1	2.1
16 ^{c)}	6.5	0.80	8.1
17 ^{c)}	7.2	0.63	11

a) Polymerization of lactone ($0.05 - 1.0 \text{ mol} \cdot \text{L}^{-1}$) was carried out using lipase PF (200 mg) catalyst in the presence of 1-octanol ($0.03 \text{ mol} \cdot \text{L}^{-1}$) in *i*-propyl ether (10 mL) at 60°C. b) Data from ref. 23. c) This study.

that the enzymatic polymerizability increased as a function of the ring size, and the large polymerizability of macrolides through lipase catalysis is mainly due to the large reaction rate ($V_{\max}$), but not to the binding abilities, *i.e.*, the reaction process of the lipase-lactone complex to the acyl-enzyme intermediate is the key step of the polymerization.

In conclusion, a 17-membered lactone was polymerized through lipase catalysis to produce the corresponding polyester. Lipases of various origins induced the polymerization, and among them lipase PF showed the highest catalytic activity toward the present polymerization. Michaelis-Menten kinetics showed that HDL had the largest enzymatic polymerizability, and difference of the polymerizability of lactones is mainly due to the conversion of the lipase-lactone complex to the acyl-enzyme intermediate.

Materials and methods. *Materials.* HDL (mp. = 33–34°C) and other lactones are commercially available reagents and stored over freshly activated type 4 molecular sieves. Lipase CA was a gift from Novo Nordisk Bioindustry, Ltd. Lipases PC and PF were donated by Amano Pharmaceutical Co. Lipase CC was purchased from Biocatalysts, Ltd. Lipases were used without further purification.

Enzymatic Polymerization. A typical run was as follows (entry 7 in Table I). 0.25 g (1.0 mmol) of HDL and 0.050 g of lipase PF were placed in a dried tube and sealed. The tube was kept on standing at 60°C for 120 h. The reaction mixture was extracted with chloroform and part of the organic solution was separated by filtration. The filtrate was analyzed by SEC for the determination of the monomer conversion and of the polymer molecular weight. The monomer conversion was calculated from the ratio of the peak areas between HDL and the polymer. The

polymer was isolated by the reprecipitation procedure (chloroform as good solvent; methanol as poor solvent).

Measurements. SEC analysis was carried out using a Tosoh SC8010 apparatus with a refractive index (RI) detector under the following conditions: TSKgel G3000H_{HR} column and chloroform eluent at a flow rate of 1.0 mL/min. The calibration curves for SEC analysis were obtained using polystyrene standards. ¹H NMR spectra were recorded on a 270 MHz JEOL EX270 spectrometer. GC analysis was carried out using a Shimadzu GC-14B apparatus equipped with an FID detector and a TC-5 column (GL Sciences).

Acknowledgement. This work was supported by a Grant-in-Aid for Specially Promoted Research (No. 08102002) from the Ministry of Education, Science, Sports and Culture of Japan. We acknowledge the gift of lipases from Amano Pharmaceutical Co. and Novo Nordisk Bioindustry, Ltd.

References

- 1) Kobayashi, S., Shoda, S., and Uyama, H. (1997) In *Catalysis in Precision Polymerization* (ed. Kobayashi, S.). John Wiley & Sons, Chichester, Chapter 8.
- 2) Kobayashi, S., Shoda, S., and Uyama, H. (1995) *Adv. Polym. Sci.* **121**, 1–30.
- 3) Ritter, H. (1993) *Trends Polym. Sci.* **1**, 171–173.
- 4) Kobayashi, S., and Uyama, H. (1998) *ACS Symp. ser.* **684**, 58–73.
- 5) Wallace, J. S., and Morrow, C. J. (1989) *J. Polym. Sci., Polym. Chem. ed.* **27**, 2553–2567.
- 6) Namekawa, S., Uyama, H., and Kobayashi, S. (1996) *Polym. J.* **28**, 730–731.
- 7) Matsumura, S., Beppu, H., Nakamura, K., Osanai, S., and Toshima, K. (1996) *Chem. Lett.*, pp. 795–796.
- 8) Svirkin, Y. Y., Xu, J., Gross, R. A., Kaplan, D. L., and Swift, G. (1996) *Macromolecules* **29**, 4591–4597.
- 9) Nobes, G. A. R., Kazlauskas, R. J., and Marchessault, R. H. (1996) *Macromolecules* **29**, 4829–4833.
- 10) Xie, W., Li, J., Chen, D., and Wang, P. G. (1997) *Macromolecules* **30**, 6997–6998.
- 11) Knani, D., Gutman, A. L., and Kohn, D. H. (1993) *J. Polym. Sci., Polym. Chem. ed.* **31**, 1221–1232.
- 12) Uyama, H., and Kobayashi, S. (1993) *Chem. Lett.*, pp. 1149–1150.
- 13) MacDonald, R. T., Pulapura, S. K., Svirkin, Y. Y., Gross, R. A., Kaplan, D. L., Akkara, J. A., Swift, G., and Wolk, S. (1995) *Macromolecules* **28**, 73–78.
- 14) Uyama, H., Takeya, K., and Kobayashi, S. (1993) *Proc. Japan Acad.* **69B**, 203–207.
- 15) Uyama, H., Takeya, K., and Kobayashi, S. (1995) *Bull. Chem. Soc. Jpn.* **68**, 56–61.
- 16) Uyama, H., Takeya, K., Hoshi, N., and Kobayashi, S. (1995) *Macromolecules* **28**, 7046–7050.

- 17) Uyama, H., Kikuchi, H., Takeya, K., and Kobayashi, S. (1996) *Acta Polymerica* **47**, 357–360.
- 18) Bisht, K. S., Henderson, L. A., Gross, R. A., Kaplan, D. L., and Swift, G. (1997): *Macromolecules* **30**, 2705–2711.
- 19) Matsumura, S., Mabuchi, K., and Toshima, K. (1997) *Macromol. Rapid Commun.* **18**, 477–482.
- 20) Matsumura, S., Tsukuda, K., and Toshima, K. (1997) *Macromolecules* **30**, 3122–3124.
- 21) Kobayashi, S., Kikuchi, H., and Uyama, H. (1997) *Macromol. Rapid Commun.* **18**, 575–579.
- 22) Bisht, K. S., Svirkin, Y. Y., Henderson, L. A., Gross, R. A., Kaplan, D. L., and Swift, G. (1997) *Macromolecules* **30**, 7735–7742.
- 23) Uyama, H., Namekawa, S., and Uyama, H. (1997) *Polym. J.* **29**, 299–301.