Extremely Efficient Catalysis of Immobilized Lipase in Ring-opening Polymerization of Lactones

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An immobilized lipase derived from *Candida antarctica* was used as catalyst for the ring-opening polymerization of lactones. A small amount of the immobilized enzyme showed the extremely efficient catalysis in the lactone polymerization. The polymerization in the presence of 1-octanol enhanced the rate of reaction.

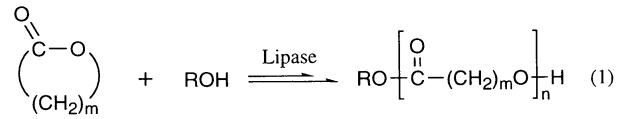
Recently, enzymatic catalysis attracts much attention as a new tool of polymer synthesis.[1] Various aliphatic polyesters have been synthesized by lipase as catalyst.[2] Lactones of various ring-sizes[3-11], lactide[12], and 6-membered cyclic carbonate[13,14] were subjected to lipase-catalyzed ring-opening polymerization under mild reaction conditions. In the polymerization of lactones using lipase derived from *Pseudomonas fluorescens* (lipase PF) as catalyst, macrocyclic lactones (12-, 13-, and 16-membered) with smaller ring-strain, which thus show lower anionic polymerizability,[15] were polymerized faster than 7-membered lactone, ε-caprolactone (ε-CL).[5-7] This is due to a favored transition state of the macrolide to open the ring.[16]

In enzyme-catalyzed reactions and polymerizations in organic solvents, a powdery enzyme is usually suspended in such media. Therefore, much amount of the enzyme is required owing to the heterogeneous reaction. For the lipase-catalyzed polymerization of lactones, we normally used the catalyst amount of 20-50 weight% for the monomer.[4-7] This amount was rather small in comparison with that reported for the enzymatic synthesis of polyesters.[17] Previously we prepared the immobilized lipase PF on Celite showing high catalytic activity toward the macrolide polymerization.[18] Only 1 weight% enzyme was enough to show similar activities as those by the powdery enzyme.

In our previous studies, the lipase-catalyzed polymerization of ε-CL proceeded slowly; only 71% of the monomer was consumed in the polymerization catalyzed by lipase PF at 60 ℃ for 10 days, although lipase PF was very active for the ε-CL polymerization among the powdery lipases.[2]

Very recently, we have found that a granular immobilized lipase derived from *Candida antarctica* (lipase CA) catalyzed the polymerization of the cyclic carbonate.[14] It is a commercial product of Novo Nordisk Bioindustry, Ltd. and the enzyme is immobilized on a macroporous acrylic resin (tradename: Novozym® 435). The present study deals with the enzymatic ring-opening polymerization of lactones catalyzed by the immobilized lipase CA.

The polymerization of lactones was carried out in bulk at 60 ℃ under argon. In this study, ε-CL, 11-undecanolide (12-



(1)

m=5 : ε-CL
m=10 : UDL
m=11 : DDL

Table 1. Lipase-catalyzed ring-opening polymerization of lactones[a]

Entry	Monomer	Enzyme		Time/h	Conv./%[b]	Mn/x10^{-3}[b]	Mw/Mn[b]
		Code	Amount/mg				
1	ε-CL	Lipase CA	1	4	16	1.8	2.7
2	ε-CL	Lipase CA	2	4	35	2.6	2.7
3	ε-CL	Lipase CA	5	4	61	3.6	3.0
4	ε-CL	Lipase CA	10	0.5	28	2.7	2.4
5	ε-CL	Lipase CA	10	1	46	3.2	2.4
6	ε-CL	Lipase CA	10	4	72	5.2	3.2
7	ε-CL	Lipase CA	10	8	91	4.0	2.3
8	ε-CL	Lipase CA	10	24	99	4.3	2.7
9	ε-CL	Lipase CA	20	4	98	5.0	2.5
10	ε-CL	Lipase PF	10	24	0		
11[c]	ε-CL	Lipase PF	50	240	71	7.0	2.2
12	UDL	Lipase CA	10	4	50	5.5	3.6
13	UDL	Lipase CA	10	24	88	4.9	3.7
14	DDL	Lipase CA	10	4	38	3.4	3.7
15	DDL	Lipase CA	10	24	59	2.8	3.4
16	DDL	Lipase PF	10	24	12	2.3	2.0
17[d]	DDL	Lipase PF	50	240	100	4.7	2.7

[a] Polymerization of lactone (1 mmol) using lipase catalyst in bulk at 60 ℃ under argon.
[b] Determined by GPC using chloroform eluent, calibrated with polystyrene standards.
[c] Data from Ref.2. [d] Data from Ref. 6.

Table 2. Lipase-CA catalyzed polymerizationof lactones in the presence of 1-octanol[a]

Entry	Monomer	[1-Octanol]$_0$/[Monomer]$_0$	Time/h	Conv./%[b]	Mn/x10^{-3}[b]	Mw/Mn[b]
1	ε-CL	0	1	46	3.2	2.4
2	ε-CL	0.05	1	62	1.8	1.8
3	ε-CL	0.1	1	72	1.4	1.7
4	ε-CL	0.2	1	78	1.0	1.5
5	DDL	0	4	38	3.4	3.7
6	DDL	0.05	4	57	2.4	2.1
7	DDL	0.1	4	67	2.3	1.9
8	DDL	0.2	4	83	1.8	1.7

[a] Polymerization of lactone (1 mmol) using lipase CA catalyst (10 mg) in bulk at 60 °C under argon.
[b] Determined by GPC using chloroform eluent, calibrated with polystyrene standards.

membered lactone, UDL), and 12-dodecanolide (13-membered lactone, DDL) were used as monomer (Eq. 1). Polymerization results are summarized in Table 1. At first, effect of the loading amount of the lipase CA was examined in the polymerization of ε-CL for 4 h. Only 1 mg of the lipase CA (less than 1 weight% for the monomer) induced the polymerization (Entry 1). The conversion increased as a function of the enzyme amount. The monomer was consumed almost quantitatively only for 4 h by using 20 mg of the lipase CA (Entry 9). The molecular weight of the resulting polymer, determined by gel permeation chromatography (GPC), was in the range of several thousands.

The polymerization of ε-CL using 10 mg of the lipase CA was monitored (Entries 4-8). The monomer conversion reached 28% for 30 min, which increased with increasing the polymerization time. After 24 h, ε-CL was quantitatively converted to the corresponding polymer. Under the similar reaction conditions, lipase PF did not induce the polymerization of ε-CL (Entry 10). These data indicate that a small amount of the lipase CA showed the extremely efficient catalysis in the polymerization of ε-CL.

UDL and DDL were also efficiently polymerized by the lipase CA catalyst (Entries 12-15). The DDL polymerization using the lipase CA catalyst proceeded faster than that by lipase PF (Entries 15 and 16). These macrolides polymerized slower than ε-CL with the lipase CA catalyst. This is in contrast to the polymerization catalyzed by lipase PF;[5,6] the macrolides polymerized faster than ε-CL by lipase PF.

We first proposed that the lipase-catalyzed polymerization of lactones proceeds via "monomer-activated mechanism",[5] which was then followed.[8] The rate-determining step is the formation of the intermediate between lipase and lactone (acyl-enzyme intermediate),[16] which we regard as enzyme-activated monomer (EM). In case of the lactone polymerization without any additives, a small amount of water, probably contained in the reaction mixture, acts as initiating species; water nucleophilically attacks the acyl carbon of EM to give an oxyacid. In the propagation stage, the terminal alcohol group of the polymer attacks EM to produce a one-unit elongated polymer chain.

When an appropriate amount of alcohol is present in the initial stage, the initiation reaction may proceed faster than that without additives since there are much more initiating species in the reaction system. Here, the enzymatic polymerization in the presence of 1-octanol has been examined (Table 2). For both monomers, the polymerization proceeded faster by the addition of the alcohol and the monomer conversion increased as a function of the alcohol concentration, whereas the molecular weight decreased by the addition of 1-octanol.

The terminal structure of the polymer (Entry 7 in Table 2) was analyzed by ^{1}H NMR spectroscopy. From the ratio of the integrated areas between a triplet peak at δ 0.9 due to methyl protons of the octyl group and a triplet peak at δ 3.6 ascribed to α-methylene protons of the terminal hydroxy group, octyl group was found to be quantitatively introduced at the polymer terminal, suggesting the possibility that end-functional polyesters are prepared by the lipase CA-catalyzed polymerization in the presence of functional alcohols.

In conclusion, the polymerization of ε-CL using a small amount of the lipase CA proceeded much faster than that of powdery lipase. The lipase CA also induced the fast polymerization of the macrolides. By the addition of 1-octanol, the polymerization rate further increased. Further studies on the polymerization of lactones using other immobilized lipases and the synthesis of end-functional polymers by the addition of alcohol are now under progress in our laboratory.

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