Precision enzymatic polymerization to polyesters with lipase catalysts

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SUMMARY: Ring-opening polymerization of lactones with different ring-size has been achieved via lipase catalysis. Small-size (4-membered) and medium-size lactones (6- and 7-membered) as well as macrolides (12-, 13-, 16-, and 17-membered) were subjected to the lipase-catalyzed polymerization. The polymerization behaviors strongly depended on the lipase origin and the ring-size of the lactones. In using *Pseudomonas* family lipases as catalyst, the polymerization of macrolides showing much lower anionic polymerizability proceeded much faster than that of ε-caprolactone. The enzymatic polymerizability of the lactones was evaluated by Michaelis-Menten kinetics. V_{max} increased as a function of the ring-size, whereas K_m values were not so different with each other. The granular immobilized lipase derived from *Candida antarctica* showed the extremely efficient catalysis in the polymerization of ε-caprolactone. Single-step synthesis of methacryl- and ω-alkenyl-type polyester macromonomers was achieved by the lipase-catalyzed polymerization of 13-membered lactone in the presence of vinyl esters acting as terminator. Lipase also catalyzed a polycondensation of dicarboxylic acid and glycol in the aqueous medium, in which the dehydration took place in water.

Introduction

Enzymatic polymerizations provide a new synthetic method of polymers whose synthesis is often difficult by conventional polymerization processes[1-3]. The enzymatic syntheses of biopolymers such as cellulose[4], chitin[5], and lignin[6] as well as non-natural polymers have been achieved under mild reaction conditions. We have systematically investigated lipase-catalyzed polymerizations of various monomer combinations to biodegradable polyesters and polycarbonate[7-9]. This article deals with our recent development of enzymatic polyester syntheses.

Lipase-catalyzed ring-opening polymerization of lactones

We have found that lipase catalysis induced ring-opening polymerization of lactones with various ring-sizes (Scheme 1). Small-size lactone (β-propiolactone (β-PL), 4-membered) was polymerized by using *Pseudomonas* family lipases as catalyst, yielding a mixture of linear and cyclic oligomers with molecular weight of several hundreds[10].

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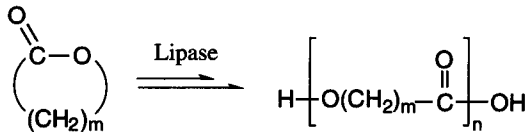
Scheme 1:



m=2 (4-Membered) : β-PL
m=4 (6-Membered) : δ-VL
m=5 (7-Membered) : ε-CL
m=7 (9-Membered) : 8-OL
m=10 (12-Membered) : UDL
m=11 (13-Membered) : DDL
m=14 (16-Membered) : PDL
m=15 (17-Membered) : HDL

Medium-size lactones, δ-valerolactone (δ-VL, 6-membered) and ε-caprolactone (ε-CL, 7-membered), were also subjected to the lipase-catalyzed polymerization[11,12]. The polymerization of these monomers took place by using lipases of different origin as catalyst, e.g., *Candida cylindracea* lipase (lipase CC), *Pseudomonas cepacia* lipase (lipase PC), *Pseudomonas fluorescens* lipase (lipase PF), and porcine pancreas lipase (PPL), which were powdery and commercially available crude enzymes. The terminal structure of the polymer was alcohol at one end and carboxylic acid at the other. In the polymerization without the enzyme or using the deactivated enzyme, which was prepared by thermal treatment at 100 ℃ in water, all the monomers were recovered unreactedly, indicating that the polymerization proceeded through the lipase catalysis.

In the polymerization of δ-VL catalyzed by these enzymes, the molecular weight of the polymer was relatively low (less than 2000). In case of the lipase-catalyzed polymerization of ε-CL, the molecular weight depended on the lipase origin; the polymerization using lipase PF catalyst at 75 ℃ produced the polymer with molecular weight of more than $1x10^4$, whereas the molecular weight was in the range of several thousands in using lipase CC or PPL catalyst under the similar reaction conditions. The polymerization of δ-VL catalyzed by lipase PF proceeded faster than that of ε-CL. 9-Membered lactone (8-octanolide, 8-OL) was also polymerized by lipase catalyst[13]. The polymerization catalyzed by lipase PC at 75 ℃ for 10 days produced the polymer with molecular weight of $1.6x10^4$.

We found that four unsubstituted macrolides, 11-undecanolide (UDL, 12-membered), 12-dodecanolide (DDL, 13-membered), 15-pentadecanolide (PDL, 16-membered), and 16-hexadecanolide (HDL, 17-membered), were polymerized by using lipase catalyst[14-17]. Polymerization results are summarized in Tab. 1. The highest molecular weight ($2.5x10^4$) was achieved in the polymerization of UDL catalyzed by lipase CC. The polymerization rate using *Pseudomonas* family lipases (lipases PC and PF) was larger than that by lipase CC or PPL.

It is well known that an active site of lipase is a serine-residue and an acyl-enzyme intermediate is involved in lipase-catalyzed reactions. The polymerization mechanism is explained as follows

Tab. 1. Enzymatic polymerization of macrolides[a)]

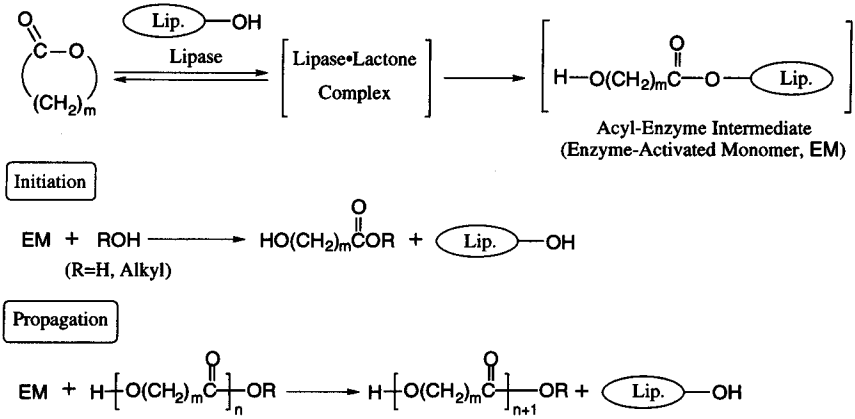
Monomer	Enzyme	Temp.	Time	Conv.[b)]	Mn[b)]	Mw/Mn[b)]
		℃	h	%		
UDL	Lipase CC	60	48	29	4400	2.9
UDL	Lipase CC	60	240	95	11700	2.4
UDL	Lipase CC	75	240	95	25200	2.2
UDL	Lipase PF	45	48	53	2800	2.5
UDL	Lipase PF	45	120	96	2900	2.5
UDL	Lipase PF	60	48	98	8500	2.4
UDL	Lipase PF	75	48	98	19500	2.5
UDL	Lipase PF	75	240	100	22800	2.6
DDL	Lipase CC	60	120	99	7300	2.3
DDL	Lipase CC	75	120	99	13000	2.8
DDL	Lipase PC	60	120	100	5600	2.3
DDL	Lipase PC	75	120	100	16400	2.4
DDL	Lipase PF	60	120	99	4000	2.5
DDL	Lipase PF	75	240	100	11400	4.3
DDL	PPL	60	120	99	3200	2.0
PDL	Lipase CC	75	240	65	16200	2.4
PDL	Lipase PF	75	120	96	7200	2.7
HDL	Lipase PC	75	120	100	5800	2.0
HDL	Lipase PF	75	120	97	5500	2.0

a) Polymerization of lactone using lipase catalyst (50 mg per 1 mmol of lactone) in bulk.
b) Determined by SEC using chloroform eluent.

(Scheme 2)[14)]. The key step is the reaction of the lactone with lipase involving the ring-opening of the lactone to give the acyl-enzyme intermediate (enzyme-activated monomer, EM). The initiation is a nucleophilic attack of water, which is probably contained in the enzyme, on the acyl carbon of the intermediate, producing ω-hydroxycarboxylic acid (n = 1). In the propagation stage, the intermediate is nucleophilically attacked by the terminal hydroxy group of a propagating polymer to give one-unit-more elongated polymer chain. Kinetic analysis showed that the rate-determining step of the over-all polymerization is the formation of the enzyme-activated monomer[18)]. Therefore, the present polymerization proceeds via a "monomer-activated mechanism".

The reactivity of cyclic compounds generally depends on the ring-size; small- and medium-size compounds show high reactivity toward the ring-opening polymerization owing to their large strain in ring. Tab. 2 summarizes dipole moment and reactivities of lactones with different ring-size[14,15)]. The dipole moment of the monomers is shown as an indication of their ring strain. The values of the macrolides are lower than that of ε-CL and close to that of an acyclic fatty acid ester

Scheme 2:



(butyl caproate). The rate constants of the macrolides in alkaline hydrolysis and anionic polymerization are much smaller than those of ε-CL. These data imply that the macrolides have much lower ring strain, and hence, show less anionic reactivity and polymerizability than ε-CL.

For the quantitative evaluation of the enzymatic polymerizability, we performed Michaelis-Menten kinetics of the polymerization (Tab. 2)[17,18]. The polymerization was carried out in the presence of 1-octanol. For all the monomers examined, linearity was observed in the Hanes-Woolf plot, indicating that the polymerization followed Michaelis-Menten kinetics. $V_{max(lactone)}$ / $K_{m(lactone)}$ 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 $V_{max(lactone)}$ / $K_{m(lactone)}$ value. $K_{m(lactone)}$ values were not so different with each other, on the other hand, $V_{max(lactone)}$ increased with increasing the ring size. These data imply 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.

Efficient catalysis of immobilized lipase in ring-opening polymerization of lactones

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. Very recently, we have found that a 6-membered cyclic carbonate was polymerized by a granular immobilized lipase derived from *Candida antarctica* (lipase CA)[19], which is immobilized on a macroporous acrylic resin. Here, the enzymatic ring-opening polymerization of lactones catalyzed by the immobilized lipase CA

Tab. 2. Dipole moments and reactivities of lactones

Lactone	Dipole moment	Rate constant		Michaelis-Menten kinetics[c]		
		Alkaline hydrolysis[a]	Propagation[b]	$K_{m(lactone)}$	$V_{max(lactone)}$	$V_{max(lactone)} / K_{m(lactone)}$
	μ	$L \cdot mol^{-1} \cdot s^{-1}$, $x10^4$	s^{-1}, $x10^3$	$mol \cdot L^{-1}$	$mol \cdot L^{-1} \cdot h^{-1}$, $x10^2$	h^{-1}, $x10^2$
δ-VL	4.22	55000	---	---	---	---
ε-CL	4.45	2550	120	0.61	0.66	1.1
UDL	1.86	3.3	2.2	0.58	0.78	1.4
DDL	1.86	6.0	15	1.1	2.3	2.1
PDL	1.86	6.5	---	0.80	6.5	8.1
HDL	---	---	---	0.63	7.2	11
Butyl caproate	1.75	8.4	---	---	---	---

[a] Alkaline: NaOH. Measured in 1,4-dioxane/water (60/40 vol%) at 0 °C.

[b] Measured using NaOMe initiator (6 mol%) in THF at 0 °C.

[c] Kinetics of the polymerization was carried out using lipase PF (200 mg) as catalyst in the presence of 1-octanol (0.03 $mol \cdot L^{-1}$) in *i*-propyl ether (10 mL) at 60 °C.

has been examined[12,20].

The polymerization of lactones was carried out in bulk at 60 °C under argon (Tab. 3). In case of the polymerization of ε-CL (1 mmol, 0.11 g), only 1 mg of 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 polymerization of ε-CL using 10 mg of 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 lipase CA showed the extremely efficient catalysis in the polymerization of ε-CL. DDL was also efficiently polymerized by lipase CA catalyst (entries 12 and 13). The DDL polymerization using lipase CA catalyst proceeded faster than that by lipase PF (entries 13 and 14). DDL polymerized slower than ε-CL with lipase CA catalyst. This is in contrast to the polymerization catalyzed by lipase PF; the macrolides polymerized faster than ε-CL by lipase PF.

Single-step synthesis of end-functionalized polyesters

Structural control of polymer terminal has been extensively studied since terminal-functionalized polymers, typically macromonomers and telechelics, are very useful prepolymers for synthesis of functional polymers. Various methodologies for synthesis of these polymers have been developed, however, most of them required elaborate and time-consuming procedures. Recently, we have achieved a single-step, convenient production of end-functionalized polyesters by lipase-catalyzed polymerization of DDL in the presence of vinyl esters[21,22]. The vinyl ester acted as terminator during the polymerization ("terminator method").

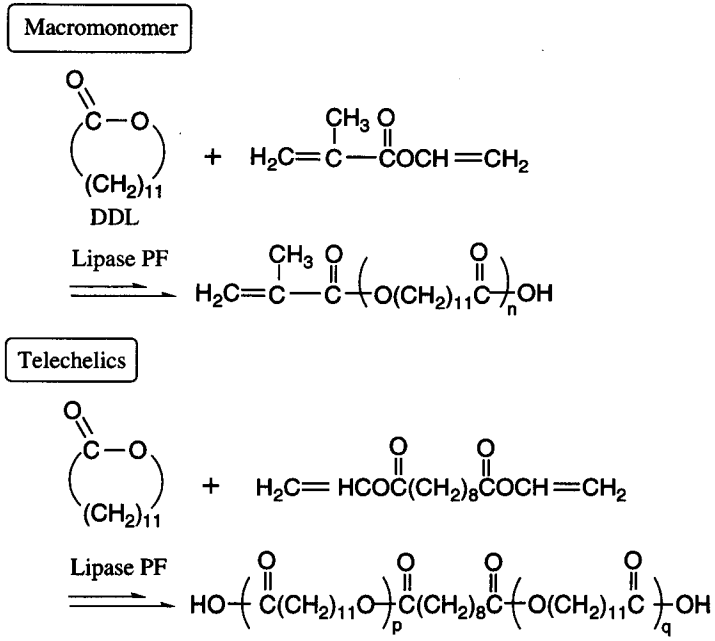
In using vinyl methacrylate (12.5 or 15 mol% based on DDL) and lipase PF as terminator and catalyst, respectively, the methacryl group was quantitatively introduced at the polymer terminal to give the methacryl-type polyester macromonomer (Scheme 3). The polymerization in the presence of vinyl 10-undecanoate produced the ω-alkenyl-type macromonomer. The macromonomer production may be explained as follows. During the polymerization of DDL, the enzyme is reacted with the vinyl ester to give the acyl-lipase intermediate, which is subjected to the reaction with the terminal hydroxy group of the lactone polymer to give the macromonomer. Furthermore, the present system can be applied to the synthesis of the telechelics having a carboxylic acid group at both ends by the addition of divinyl sebacate in the reaction mixture.

Tab.3. Ring-opening polymerization of lactones using the immobilized lipase CA[a)]

Entry	Monomer	Enzyme		Time	Conv.[b)]	M_n[b)]	M_w/M_n[b)]
		Code	Amount				
			mg	h	%		
1	ε-CL	Lipase CA	1	4	16	1800	2.7
2	ε-CL	Lipase CA	2	4	35	2600	2.7
3	ε-CL	Lipase CA	5	4	61	3600	3.0
4	ε-CL	Lipase CA	10	0.5	28	2700	2.4
5	ε-CL	Lipase CA	10	1	46	3200	2.4
6	ε-CL	Lipase CA	10	4	72	5200	3.2
7	ε-CL	Lipase CA	10	8	91	4000	2.3
8	ε-CL	Lipase CA	10	24	99	4300	2.7
9	ε-CL	Lipase CA	20	4	98	5000	2.5
10	ε-CL	Lipase PF	10	24	0		
11	ε-CL	Lipase PF	50	240	71	7000	2.2
12	DDL	Lipase CA	10	4	38	3400	3.7
13	DDL	Lipase CA	10	24	59	2800	3.4
14	DDL	Lipase PF	10	24	12	2300	2.0
15	DDL	Lipase PF	50	240	100	4700	2.7

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

Scheme 3:



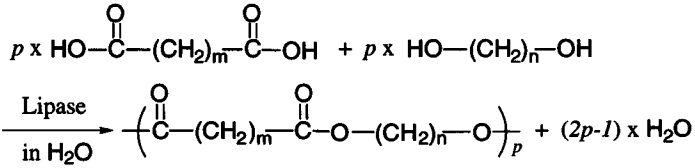
Polyester macromonomers were also synthesized by enzymatic ring-opening polymerization of DDL in the presence of a functional alcohol[23]. The alcohol initiated the ring-opening polymerization of lactones using lipase CA as catalyst to introduce the alcohol moiety at the polymer terminal ("initiator method"). In the polymerization of DDL employing 2-hydroxyethyl methacrylate as initiator, the methacryl group was quantitatively introduced at the polymer terminal, yielding the methacryl-type polyester macromonomer. ω-Alkenyl-type macromonomers were synthesized by using 5-hexen-1-ol or 5-hexyn-1-ol as initiator.

Dehydration polymerization in water

A dehydration reaction is generally realized in non-aqueous media. Since a product water of the dehydration is in equilibrium with starting materials, a solvent water disfavors the dehydration to proceed in an aqueous medium due to the law of mass action. On the other hand, we have found that lipase catalysis provided a dehydration polymerization of a dicarboxylic acid and glycol in water (Scheme 4)[24]. The view of dehydration in an aqueous medium is a new aspect in organic chemistry.

The polymerization of sebacic acid and 1,8-octanediol using lipase PC as catalyst was performed in distilled water at 45 ℃ for 24 h to give the corresponding polyester with molecular weight of 1600 in 43 % yield. Lipases CA, CC, and PF also showed high catalytic activity. Both

Scheme 4:



monomers were recovered unchanged in the absence of enzyme (control experiment). These results indicate that the present dehydration polymerization proceeded through enzyme catalysis in the aqueous medium. The chain length of both monomers strongly affected the polymerization behavior; the combination of the monomers with appropriate hydrophobicity was favored for the polymer formation. A similar tendency is observed in the lipase-catalyzed ring-opening polymerization of lactones in an aqueous medium[25].

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