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Headline Articles

Enzymatic Ring-Opening Polymerization of Lactones to Polyesters by Lipase Catalyst: Unusually High Reactivity of Macrolides

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Enzymatic ring-opening polymerization of macrolides was carried out by using various lipases as catalysts. The monomers used in this study were 11-undecanolide (12-membered, UDL) and 15-pentadecanolide (16-membered, PDL). Among the enzymes examined, lipases derived from *Pseudomonas fluorescens* (lipase P) and from *Candida cylindracea* (lipase B) gave polyUDL with high molecular weight in a high yield. From ^{1}H and ^{13}C NMR analysis, the polymer was found to possess the terminal structure of a carboxylic acid group at one end and a hydroxyl group at the other. The rate of the UDL polymerization using lipase P was larger than that using lipase B, whereas the polymerization of UDL using lipase B produced the polymer of higher molecular weight in comparison with that obtained by using lipase P. Lipases from *Pseudomonas* sp. and porcine pancreas showed a catalytic activity for the polymerization of UDL. PDL was also polymerized by lipase catalyst to give the corresponding polyester. The enzymatic polymerizations of UDL and PDL have been compared with that of 6-hexanolide (ε-caprolactone, ε-CL). The polymerization of the macrolides using lipase P proceeded much faster than that of ε-CL. This is probably due to the strong recognition of the macrolides by the lipase catalyst.

Enzyme-catalyzed reactions in organic solvents have been increasingly important in organic synthesis.[1] Polymerizations catalyzed by enzymes (enzymatic polymerizations) received little attention until several years ago because such specific properties of enzymes had not been fully utilized in most of these polymerizations.[2,3] Recently, however, the synthesis of cellulose via a nonbiosynthetic path has successfully been achieved by the enzymatic polymerization of β-cellobiosyl fluoride, using cellulase as catalyst.[4] An optical active polyester was synthesized by the lipase-catalyzed polymerization of an epoxide-containing diester with diol monomers.[5] Very recently, we have paid attention to the high reactivity of enol esters by lipase catalyst and found that aliphatic polyesters were obtained by the enzymatic polymerization of divinyl adipate with a glycol under mild reaction conditions.[6] Up to now, almost all the enzymatic polymerizations were of polycondensation type. Very recently, two novel types of the enzymatic polymerization involving ring-opening of a monomer using a lipase catalyst have been reported; one is a ring-opening polymerization and copolymerization of lactones[7—9] and the other is a poly(addition-condensation) between a cyclic acid anhydride and a glycol.[10]

Small- and medium-size lactones (4, 6, and 7-membered) are polymerized by various catalysts under mild conditions owing to the large strains in their rings.[11] On the other hand, there has been less attention paid to the polymerization of macrolides, macrocyclic esters. Very recently, anionic polymerization of 12- and 13-membered lactones has been reported.[12] The polymerizability of these monomers was much lower than that of ε-caprolactone (ε-CL), because the ring strain is lower than that of ε-CL. The present paper describes the enzymatic ring-opening polymerization of macrolides. In this study, 11-undecanolide (12-membered, UDL) and 15-pentadecanolide (16-membered, PDL) were employed as monomers. These macrolide monomers have been enzymatically polymerized much faster than ε-CL, contrary to the order of the ring strains of lactone monomers.

Experimental

Materials. UDL was purchased from Lancaster and

PDL from Tokyo Kasei Co. These monomers were purified by the addition of freshly activated type 4 molecular sieves. Lipases were employed without further purification. Lipase B from *Candida cylindracea*, lipase D from *Rhizopus delemer*, and lipase P from *Pseudomonas fluorescens* were purchased from Cosmo Bio. Lipase derived from porcine pancreas was purchased from Sigma. Lipase A from *Aspergillus niger*, lipase AY from *Candida rugosa*, lipase F from *Rhizopus* sp., and lipase PS from *Pseudomonas* sp. were donated by Amano Pharmaceutical Co. Lipase J from *Rhizopus japonicus* was obtained from Nagase Seikagaku Co. Phospholipase from porcine pancreas (PLA2) and esterase from porcine liver (EST) were gifts from Kyowa Hakko Co.

Enzymatic Polymerization. A typical run was as follows (Entry 7 in Table 1): 0.11 g (0.60 mmol) of UDL and 0.030 g of lipase P were placed in a dried test tube and sealed. The tube was kept 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 gel permeation chromatography (GPC) 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 the polymer and UDL. The polyester was isolated by the reprecipitation procedure (chloroform as good solvent; methanol as poor solvent).

Measurements. GPC analysis was carried out using a TOSO SC8010 apparatus with a refractive index (RI) detector under the following conditions: TSKgel G3000H_{HR} or Gelpack GL-A150 column and chloroform eluent at a flow rate of 1.0 mL min^{-1}. The calibration curves for GPC analysis were obtained using polystyrene standards. ^{1}H and ^{13}C NMR spectra were recorded on a 250 MHz Bruker AC-250T spectrometer.

Results and Discussion

Enzyme Screening. At first, various types of lipases with different origins were used as catalysts for the polymerization of UDL, in order to search for an enzyme of high activity for the polymerization. UDL was polymerized in bulk at 60 °C for 120 h. Lipases used in this study were derived from *Aspergillus niger* (lipase A), *Candida rugosa* (lipase AY), *Candida cylindracea* (lipase B), *Rhizopus delemar* (lipase D), *Rhizopus* sp. (lipase F), *Rhizopus javanicus* (lipase J), *Pseudomonas fluorescens* (lipase P), *Pseudomonas* sp. (lipase PS), and porcine pancreas (PPL). Phospholipase from porcine pancreas (PLA2) and esterase from porcine liver (EST) were also used as catalysts. All the enzymes were fine powders and were dispersed in the monomer. The polymerization results are shown in Table 1.

Table 1. Enzymatic Ring-Opening Polymerization of UDL Catalyzed by Lipase of Different Origin[a)]

Entry	Catalyst	Conv.[b)]	M_n[b)]	M_w/M_n[b)]
		%		
1	Lipase A	40	1200	1.3
2	Lipase AY	0	—	—
3	Lipase B	84	9400	2.1
4	Lipase D	0	—	—
5	Lipase F	0	—	—
6	Lipase J	0	—	—
7	Lipase P	98	8400	2.5
8	Lipase PS	100	5700	2.3
9	PPL	85	5800	1.8
10	PLA2	0	—	—
11	EST	0	—	—
12	—	0	—	—

a) Polymerization in bulk at 60 °C for 120 h. b) Determined by GPC.

UDL was quantitatively consumed by lipase P or PS catalyst to produce the polymer. The molecular weight of the polymer obtained by using lipase P was larger than that by lipase PS. We have already reported that lipase P was the most active for the enzymatic ring-opening polymerization of ε-CL.[7] Therefore, the lipase P catalyst was found to exhibit a high activity for the polymerization of the macrolide as well as ε-CL. The polymerization using lipase B or PPL catalyst also afforded the polymer in high yields. It is to be noted that the polymer of the highest molecular weight was obtained by lipase B catalyst. The activity of lipase A was not high; the polymerization catalyzed by lipase A produced an oligomer in low yields. On the other hand, lipases AY, D, F, and J, PLA2, and EST were not active for the polymerization. The polymerization did not take place without the enzyme (control experiment). These data indicate that the polymerization proceeds via enzyme catalysis and that the polymerization behavior very much depends upon the origin of the enzyme.[13]

Enzymatic Polymerization of UDL Catalyzed by Lipases P and B. As shown in Table 1, lipases B and P exhibited high activity for the polymerization. Then, detailed polymerization behaviors using lipases P and B were investigated. Figure 1 shows time-conversion curves in the polymerization under different reaction conditions. More than 90% conversion of UDL was attained in the polymerization by lipase P at 75 °C within only 24 h. The polymerization rate increased with increasing the polymerization temperature.

Table 2 shows the polymerization results using lipase P catalyst. Only oligomeric compounds were obtained in a low yield in the polymerization at 30 °C for 240 h (Entry 1). Above the temperature of 45 °C, more than 95% of the monomer was consumed within 120 h. The higher the polymerization temperature in the range of the temperature from 45 to 75 °C, the larger the molecular weight. As the polymerization proceeded at each polymerization temperature, the molecular weight increased.

The enzymatic polymerization of UDL catalyzed by lipase B under various conditions has been performed (Table 2). The polymerization at 30 °C for 240 h gave only oligomers with the molecular weight of 1.1×10^3

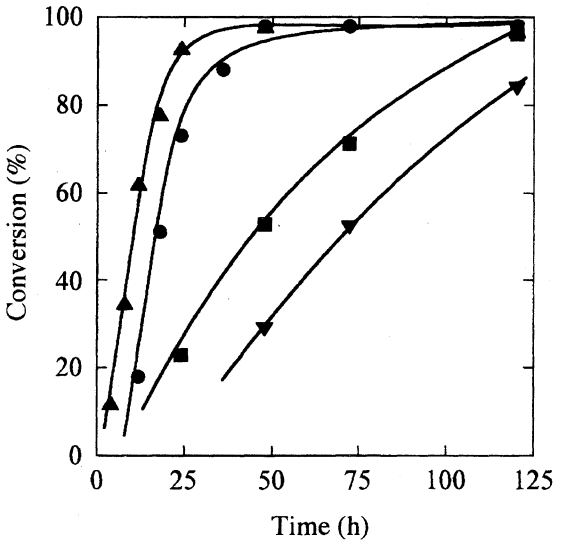


Fig. 1. Time-conversion curves in the enzymatic ring-opening polymerization of UDL under different reaction conditions: by lipase P at 45 °C (■); by lipase P at 60 °C (●); by lipase P at 75 °C (▲); by lipase B at 60 °C (▼).

Table 2. Enzymatic Ring-Opening Polymerization of UDL Using Lipases P and B as Catalyst[a)]

Entry	Catalyst	Temp °C	Time h	Conv. %	M_n[b)]	M_w/M_n[b)]
1	Lipase P	30	240	25	1100	1.4
2	Lipase P	45	24	23	2300	2.7
3	Lipase P	45	48	53	2800	2.5
4	Lipase P	45	120	96	2900	2.5
5	Lipase P	60	12	18	3600	3.0
6	Lipase P	60	24	73	6000	2.5
7	Lipase P	60	48	98	8500	2.4
8	Lipase P	75	8	35	3300	2.5
9	Lipase P	75	18	78	7400	2.7
10	Lipase P	75	48	98	19500	2.5
11	Lipase B	30	240	14	1100	1.4
12	Lipase B	45	120	35	4000	2.2
13	Lipase B	60	48	29	4400	2.9
14	Lipase B	60	120	84	9400	2.1
15	Lipase B	60	240	95	11600	2.4
16	Lipase B	75	120	94	21900	2.3

a) Polymerization in bulk. b) Determined by GPC.

in low yields. As the polymerization temperature increased, both the monomer conversion and the molecular weight of the polymer increased. The polymerization catalyzed by lipase B produced the polymer with molecular weight higher than 2×10^4, which is so far the largest value of the molecular weight of a polyester obtained by enzymatic polymerizations, and larger than that in the anionic polymerization of UDL catalyzed by sodium methoxide ($M_n=7.3\times10^3$).[12] In comparison with the polymerization behavior catalyzed by lipase P, the polymerization rate enormously decreased by lipase B catalyst (Fig. 1), indicating that lipase P recognizes UDL more strongly than lipase B. The molecular weight of the polymer obtained by lipase B catalyst was somewhat larger than that obtained by lipase P.

Terminal Structure of Polyester. In order to examine the structure of terminal groups, ^{13}C and ^1H NMR analyses of the polymer (Entry 4 in Table 2) were performed. In the ^{13}C NMR spectrum, there are three small characteristic peaks (δ 178, 63, and 33) besides the main peaks of polyUDL (Fig. 2): a peak at δ 178 (peak A) is due to the carbon of carboxylic acid, a peak at δ 63 (peak B) ascribed to the α-methylene carbon of hydroxyl group, and a peak at δ 33 (peak C) due to the methylene carbon adjacent to the carboxylic acid group. In ^1H NMR spectrum, a small triplet peak ascribed to the α-methylene protons of the hydroxyl groups is observed at δ 3.6, in addition to the main peaks of polyUDL. From the integrated area of this peak and the peak due to the α-methylene protons of $C(=O)OCH_2C$ (δ 4.0) of the polymer main chain, the molecular weight was calculated as 2.7×10^3, which is close to that determined by GPC. These data support the terminal structure of the polymer bearing a carboxylic acid group at one end and a hydroxyl group at the other (Scheme 1).[7]

Enzymatic Polymerization of PDL. A 16-membered lactone, PDL, was also polymerized by lipase catalyst to produce the corresponding polyesters (Table 3).

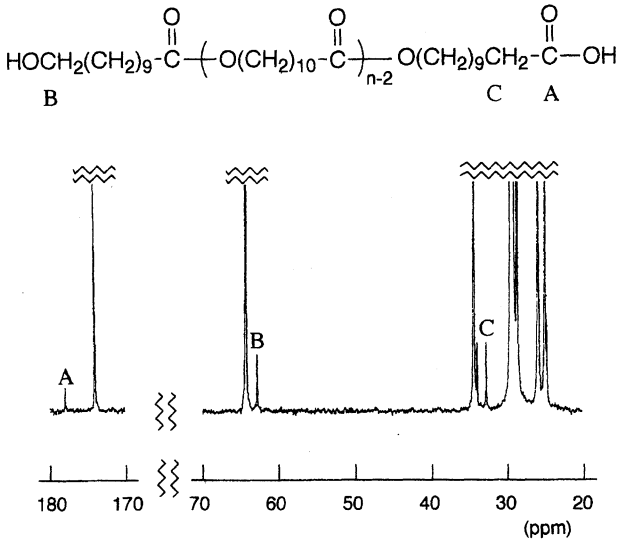
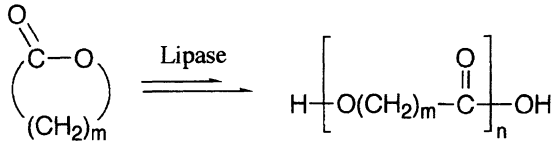


Fig. 2. ^{13}C NMR spectrum of the polymer (Entry 4 in Table 2) in $CDCl_3$.



m=10 : UDL
m=14 : PDL

Scheme 1.

Table 3. Enzymatic Ring-Opening Polymerization of PDL Catalyzed by Lipase

Entry	Catalyst	Temp	Time	Conv.	$M_n^{b)}$	$M_w/M_n^{b)}$
		°C	h	%		
1	Lipase P	45	240	8	1100	1.1
2	Lipase P	60	4	35	2600	1.8
3	Lipase P	60	48	73	2700	2.3
4	Lipase P	60	120	94	2700	2.2
5	Lipase P	75	240	100	6400	2.4
6	Lipase B	60	120	35	5800	2.8

a) Polymerization in bulk. b) Determined by GPC.

The polymerization using lipase P catalyst at 45 °C scarcely proceeded (Entry 1). On the other hand, the higher temperature induced the lipase P-catalyzed polymerization. In the polymerization using by lipase P at 60 °C, the molecular weight was almost the same after different reaction time (Entries 2—4). The higher the polymerization temperature, the higher the molecular weight. The polymerization using lipase B proceeded much more slowly than that using lipase P (Entry 6), but the resulting polymer possessed higher molecular weight.

Comparison of Lipase-Catalyzed Polymerization Behaviors between Macrolides and ε-CL. Reactivity of cyclic compounds generally depends on the ring size; the ring strain of small and moderate ring-size compounds is large, and hence, they show high reactivity toward the ring-opening reactions. Huisgen and Ott have measured dipole moments and alkaline hydrolysis rates of various lactones.[14] Dipole moment values of ε-CL, UDL, and PDL are 4.45, 1.86, and 1.86 (D, 1 D=3.33564×10^{-30} C m), respectively. The value of the macrolides is close to that of butyl hexanoate (1.79 D). The hydrolysis rate was measured with NaOH in 1,4-dioxane/water at 0 °C. These macrolides were 300 times less reactive toward the alkaline than ε-CL and exhibited even lower reactivity toward the alkaline than butyl hexanoate. Very recently, it has been reported that the propagation rate of UDL in the anionic polymerization using sodium methoxide initiator was 50 times less than that of ε-CL.[12] These data suggest that the ring-strain of these macrolides is much smaller than that of ε-CL; hence, these macrolides show less reactivity of ring-opening polymerization than ε-CL.

Lipase-catalyzed polymerization behaviors of UDL and PDL have been compared with those of ε-CL. Figure 3 shows time-conversion curves in the polymerization of ε-CL, UDL, and PDL catalyzed by lipase P at 60 °C. It is striking that the polymerization of the macrolides proceeded much faster than that of ε-CL. The initial rate of the polymerization of PDL was somewhat larger than that of UDL. These data suggest that lipase P shows much higher catalytic activity toward the macrolides than does with ε-CL. This tendency is opposite to the ring strains of these compounds.

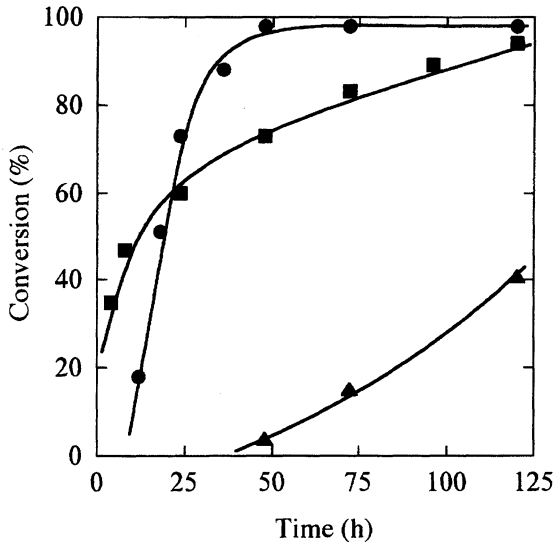


Fig. 3. Time–conversion curves in the polymerization of lactones catalyzed by lipase P at 60 °C: UDL (●); PDL (■); ε-CL (▲).

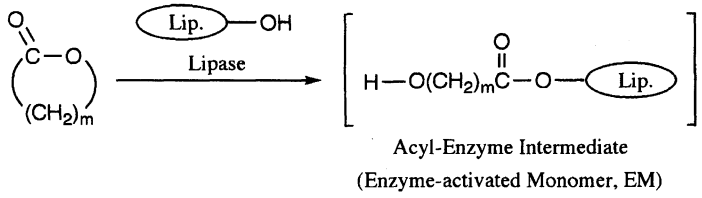
The polymerization results of UDL and ε-CL catalyzed by lipases B and P are compared in Table 4. The polymerization of ε-CL using lipase B proceeded faster than that catalyzed by lipase P, whereas the molecular weight of the polymer obtained by lipase B is much lower than that by lipase P.[7] In case of the polymerization of UDL, the opposite tendency was observed; the polymerization rate using lipase P was much faster than that using lipase B (Fig. 1) and the molecular weight of the polymer obtained by lipase P catalyst was slightly lower than that by lipase B. In the polymerization catalyzed by lipase B, the molecular weight of polyUDL was much larger than that of poly(ε-CL).

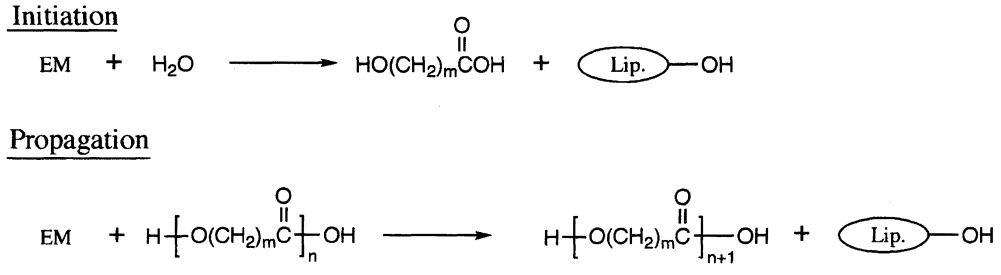
Polymerization Mechanism. Lipase-catalyzed reactions such as hydrolysis, esterification, and transesterification are well known to proceed via an acyl-enzyme intermediate.[15] The present polymerization may be explained by considering the following reactions as the principal reaction course (Scheme 2).[17] Lipase is the enzyme which catalyzes the hydrolysis of a fatty oil to a higher fatty acid and glycerol; its catalytic site of hydrolysis is believed to be a serine-residue assisted by an imidazol moiety.[15] The key step is the reaction of the macrolide with lipase involving the ring-opening of the macrolide to produce the acyl-enzyme intermediate (enzyme-activated monomer, EM). The initiation is a nucleophilic attack of water onto the acyl carbon of the intermediate to produce ω-hydroxycarboxylic acid (n=1). In the propagation stage, the intermediate is nucleophilically attacked by the terminal hydroxyl group of the polymer to produce a one-unit-more elongated polymer chain. Therefore, the present polymerization is assumed to proceed via "monomer-activated mechanism". The rate-determining step of the over-all polymerization is probably the above key step of produc-

Table 4. Comparison of the Lipase-Catalyzed Polymerization Behaviors between UDL and ε-CL[a)]

Polymerization		PolyUDL			Poly(ε-CL)[b)]		
Catalyst	Temp	Conv.[c)]	M_n[c)]	M_w/M_n[c)]	Conv.[c)]	M_n[c)]	M_w/M_n[c)]
	°C	%			%		
Lipase B	30	14	1100	1.4	41	550	1.2
Lipase B	45	95	4300	2.1	77	770	1.3
Lipase B	60	95	12000	2.2	90	1900	2.0
Lipase B	75	95	25000	2.2	98	1800	2.3
Lipase P	30	25	1100	1.4	8	1100	1.4
Lipase P	45	99	2800	2.5	40	3400	2.5
Lipase P	60	99	8300	2.6	85	7000	2.2
Lipase P	75	100	23000	2.6	92	7700	2.4

a) Polymerization in bulk for 240 h. b) Data from Refs. 7 and 16. c) Determined by GPC.





Scheme 2.

ing the acyl-enzyme intermediate, where the recognition of the monomer by the lipase enzyme is operative. Namely, the macrolides are more readily recognized and activated by the enzyme than a smaller ring-size lactone like ε-CL, because the shape of the macrolides is rather close to that of the aliphatic fatty oil compared with that of the smaller lactones.

Conclusion. The enzymatic ring-opening polymerization of the macrolides was performed under the mild conditions to give quantitatively the corresponding polyester of higher molecular weight than that obtained by the anionic polymerization. In the lipase-catalyzed polymerization, UDL and PDL were more reactive than ε-CL, the tendency of which is the reverse of the ring strain and of the anionic polymerizability of these cyclic monomers. This unusual phenomenon is due to the specificity in the enzymatic catalysis. Further investigations on the mechanism of the present polymerization and the applications of the resulting polyesters are now in progress.

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