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Enzymatic Ring-Opening Polymerization of Lactones by Lipase Catalyst: Mechanistic Aspects

Shiro Kobayashi

Summary: Mechanistic aspects of lipase-catalyzed ring-opening polymerization (ROP) of lactones to give polyesters are discussed from accumulated experimental data and new insight. Comparison of the ROP reactivity by lipase catalyst with the anionic ROP reactivity by a metal-catalyst clearly demonstrates the characteristics of lipase catalysis; the larger ring-sized monomers with lower ring strain showed higher polymerizability than medium ring-sized ones, in contrast to the anionic ROP showing the reverse direction where the ring strain of monomer is operative. The enzyme-catalysis involves an acyl-enzyme intermediate formation as a key-step. From the copolymerization results a new mechanism is proposed, that involves the formation of the acyl-enzyme intermediate (acylation step) and/or the nucleophilic attack of the propagationg alcohol end to the carbonyl carbon of the intermediate to open the monomer ring (deacylation step) as the rate-determining step. The structure of the propagating alcohol end (primary or secondary) affects much on which step is more operative.

Keywords: catalysis; lactone; lipase enzyme; reaction mechanism; ring-opening polymerization

Introduction

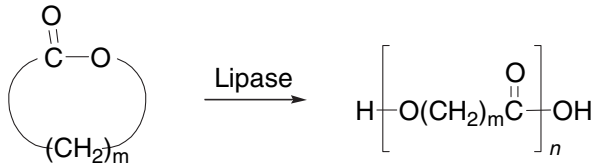
Synthesis of aliphatic polyesters had been achieved by chemical processes and by fermentation. Enzyme-catalyzed methods provided another way to the snythesis. Ring-opening polymerization (ROP) of lactones by lipase catalyst to the polyesters was first reported in 1993 by two independent groups, our group[1,2] and Knani et al.[3] The research of this field sparked for this decade since then.[4] Lipase is an enzyme which catalyses the hydrolysis of fatty acid esters normally in an aqueous environment in living systems. However, lipase is sometimes stable in organic solvents and can be used as catalyst for esterifications and transesterifications.[5] The ring-opening catalysis utilizes such specificity of lipase.[4,6] In addition to ROP, the polyesters can be produced with lipase catalysis via polycondensations of dicarboxylic acids and glycols, oxyacids, and their derivatives.[4,7] The present paper focuses on the mechanistic aspects of the lipase-catalyzed ROP of lactones, particularly the ring-opening reactivity of various ring-sized lactones, in comparison with that of conventional catalysts and the rate-determining step of the ROP from the copolymerization results.

Department of Materials Chemistry, Graduate School of Engineering, Kyoto University, Kyoto 615-8510, Japan
Present address: R & D Center for Bio-based Materials, Kyoto Institute of Technology, Kyoto 606-8585, Japan
Fax: (+81)75 724 7688
E-mail: kobayash@kit.ac.jp

Ring-Opening Polymerizability

The following lactone monomers have been investigated (Scheme 1):

As to the ROP reactivity of lactones, unsubstituted monomers of different ring-size were examined with lipase catalyst.[8] Then, we first found that macrolides of 12- and 16-membered lactones (UDL and PDL, respectively) showed a much higher



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

Scheme 1.

reactivity than ε-caprolactone (ε-CL, 7-membered) as qualitatively shown in Figure 1. Here, lipase *Pseudomonas fluorescens* (lipase PF) was used as catalyst. This result explored us to examine further on the ROP reactivity of other lactones, because it was already known that in the anionic ROP of lactones the reactivity is governed by the ring strain, suggesting the higher reactivity of ε-CL.

The polymerization mechanism of the ROP of lactones was first proposed in the same paper, which involves an acyl-enzyme intermediate (enzyme-activated monomer, EM) as shown in Scheme 2.[8] It offers a ''monomer-activated mechanism'', in contrast to a ''active-chain end mechanism'' of the conventional anionic ROP.

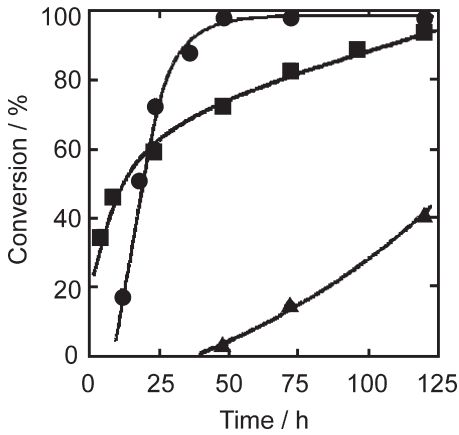
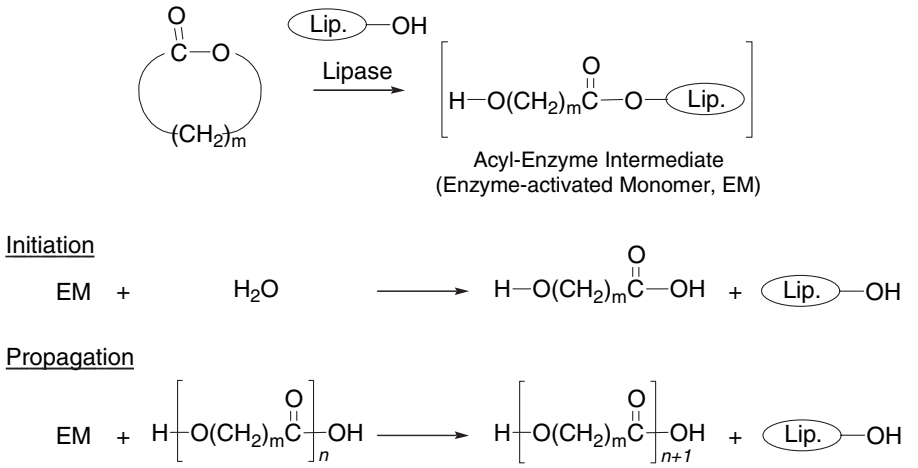


Figure 1.
Time-conversion curves in the polymerization of lactones catalyzed by lipase PF at 60 °C: UDL (●); PDL (■); ε-CL (▲).

Since the polymerization was found to obey the Michalis-Menten kinetic analysis, the polymerizability of various lactones was quantitatively studied according to the analysis to determine the relative reactivity.[9,10] The results carried out by lipase PF catalyst are summarized in Table 1,[11] together with dipole moment values and rate-constants of alkaline hydrolysis as well as anionic propagation. The kinetic analysis was made based on the assumption that the rate-determining step of the over-all polymerization reaction is the formation of the acyl-enzyme intermediate (EM).

The dipole moment value of the monomers is taken 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 (butyl caproate). The reactivity of cyclic compounds generally depends on the ring-size; small- and medium-size compounds show higher reactivity owing to their larger strain in ring in comparison with macrolides (large sized). In fact, the rate constants of the macrolides in alkaline hydrolysis and in 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.

In the enzyme-catalyzed polymerization, however, medium ring-size monomers (6 and 7 membered) are about one-magnitude less reactive than macrolide monomers (10 – 17 membered); the larger the ring-size the higher the polymerizabil-



Scheme 2.

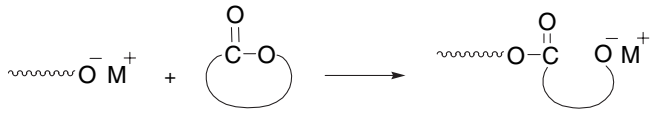
ity. For the formation of the reactive acyl-lipase intermediate, two stages are involved: the first stage is the binding of monomer (lipase-lactone complex formation) and the second stage the ring-opening of the complex to give the intermediate. We already pointed out that the second stage is rather operative in rate, with comparing the values of the binding constant (K_m) and the rate constant (V_{max}) in the Michaelis-Menten kinetics analysis. The larger ring monomer is more readily recognized and ring-opened by lipase catalyst than the smaller sized one, because lipase enzyme inherently catalyzes the hydrolysis of hydrophobic higher fatty acid esters *in vivo*, and hence, prefers *in vitro* also to recognize a hydrophobic molecule like a macrolide and to open its ring in the complex leading to the intermediate more readily than a less hydrophobic molecule like an ε-CL. This situation was explained by the geometrical factor of the lipase-lactone complex.

The mechanism of anionic ROP of lactones have been extensively studied for many years and well established.[12] The

Table 1.
Comparison in Dipole Moments and Reactivities of Various Unsubstituted Lactones

Lactones (ring-size)	Dipole moments (C · m)	Rate constants		Relative rate of polymerization	
		alkaline hydrolysis[a] ($L \cdot mol^{-1} \cdot s^{-1}$, $\times 10^4$)	propagation[b] (s^{-1}, $\times 10^3$)	enzymatic polymerization[c]	anionic polymerization[d]
δ-VL (6)	4.22	55,000	—	—	2,500
ε-CL (7)	4.45	2,550	120	0.10	330
8-OL (9)	—	—	—	—	21
UDL (12)	1.86	3.3	2.2	0.13	0.9
DDL (13)	1.86	6.0	15	0.19	1.0
PDL (16)	1.86	6.5	—	0.74	0.9
HDL (17)	—	—	—	1.00	1.0
butyl caproate	1.75	8.4	—	—	—

[a] Alkaline: NaOH. Measured in 1,4-dioxane/water at 0 °C.
[b] Measured using NaOMe initiator in THF at 0 °C.
[c] Kinetics of the polymerization was carried out using lipase PF as catalyst in the presence of 1-octanol in *i*-propyl ether (10 mL) at 60 °C.
[d] $[Zn(Oct)_2]_0 = [BuOH]_0 = 0.28$ mol · L^{-1} with bulk polymerization carried out at 100 °C.[13]



Scheme 3.

propagation step is of S_N2 type reaction and is generally given in Scheme 3, that determines the rate of overall-reaction: where M^+ is a counter-cation like Al, Zn, Sn and ammonium, and the propagation oxy-anion attacks nucleophilically onto the carbonyl carbon of the monomer to open the ring. In order to compare the polymerizability of these lactone monomers in enzymatic polymerization and anionic polymerization, Zn-catalyzed ROP was systematically examined.[13] The kinetic studies were performed by using zinc octoate, $Zn(Oct)_2$, as catalyst. Some results are shown in Figure 2. The ROP reactivity is of big difference depending on the ring-size. Quantitative relative reactivities are given in Table 1, where the ring strain is operative; the smaller the ring, the higher the polymerizabirity. This reactivity is reverse to that of lipase catalysis, which is attributed to the difference in mechanism of polymerizations by the two catalysts, a lipase enzyme and a Zn compound.

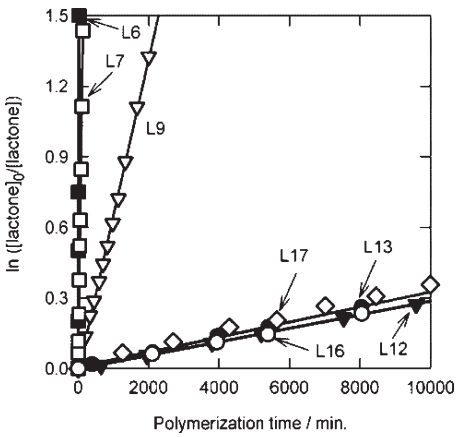
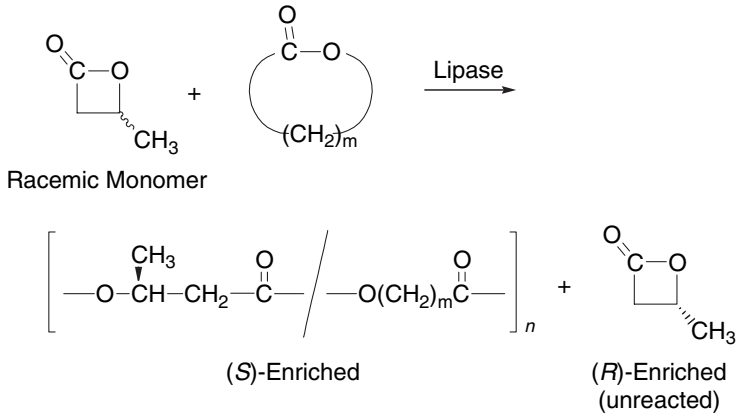


Figure 2.
Kinetics of the $Zn(Oct)_2$ -catalyzed ROP of seven lactones. In the figure, Ln of n denotes the ring-size of the monomer.

Rate-Determining Step in Ring-Opening Polymerization

We have been developing lipase-catalyzed ring-opening polymerization and copolymerization of substituted and unsubstituted lactones for polyester synthesis.[2,4,10,14–16] Lipase PC (*Pseudomonas cepacia* lipase) induced an enantioselective polymerization of a substituted 7-membered lactone, (*R*)- and (*S*)-3-methyl-4-oxa-6-hexanolides, giving rise to optically active polyesters. With the lipase catalysis, *S*-isomer showed seven times higher reactivity than *R*-isomer.[10]

Lipase CA (*Candida antarctica* lipase) catalyzed copolymerization of a racemic lactone (methyl substituted) with an achiral lactone (unsubstituted) was investigated.[16] The reaction induced an enantioselective copolymerization of the racemic monomer (Scheme 4). From the viewpoint of propagation, there are four different diads for the copolymer formation. For example, the copolymerization of β-butyrolactone (β-BL, four membered) with 12-dodecanolide involves four modes, which was proven by ^{13}C NMR spectrum (Figure 3). Four peaks (A, B, C, and D) were assigned to the respective diad and their intensity ratio is approximately 10 : 6 : 4 : 0, respectively. The corresponding four elementary propagations are given in Figure 4, showing the steps where an acyl-enzyme intermediate is attacked by a propagating end of hydroxyl group. If the rate-determining step of the lipase-catalyzed ROP is the formation of an acyl-enzyme intermediate because the subsequent reactions are very fast due to a high reactivity of the acyl-enzyme intermediate, the formation of the diad A and C and of the diads B and D should be the same in amount; however, the results are different;



Scheme 4.

they are 10 : 4 and 6 : 0, respectively. This suggests that the reaction steps of Figure 4 involve a propagating end of sterically two different structures (primary and secondary alcohols), and hence, greatly affect the overall polymerization. Similar results were obtained for the copolymerization of δ-CL (6-membered) with 12-dodecanolide, and also other binary copolymerizations.[16]

Now, the present author considers as follows; these observations lead to a mechanism of the lipase-catalyzed ROP that the formation of the acyl-enzyme intermediate (acylation step) and/or the subsequent reactions of the intermediate (deacylation step) are operative for the rate-determining step. In particular, the deacylation step becomes more important when the propagating alcohol end is of sterically hindered structure. Enantiose-

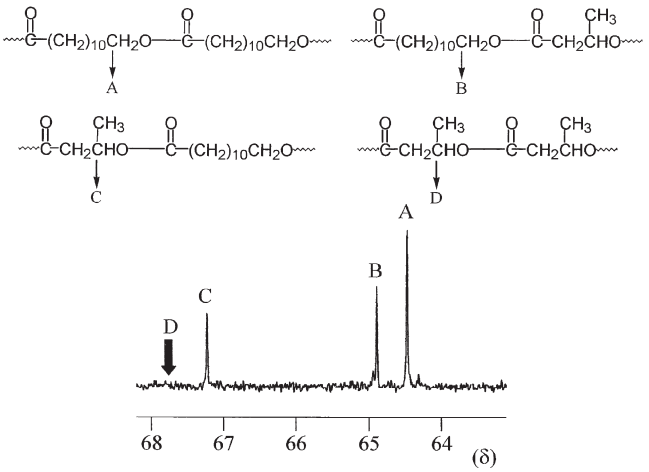


Figure 3.
^{13}C NMR four peaks due to *C*-O signals and their diad sequences of the copolymer from β-butyrolactone and 12-dodecanolide.

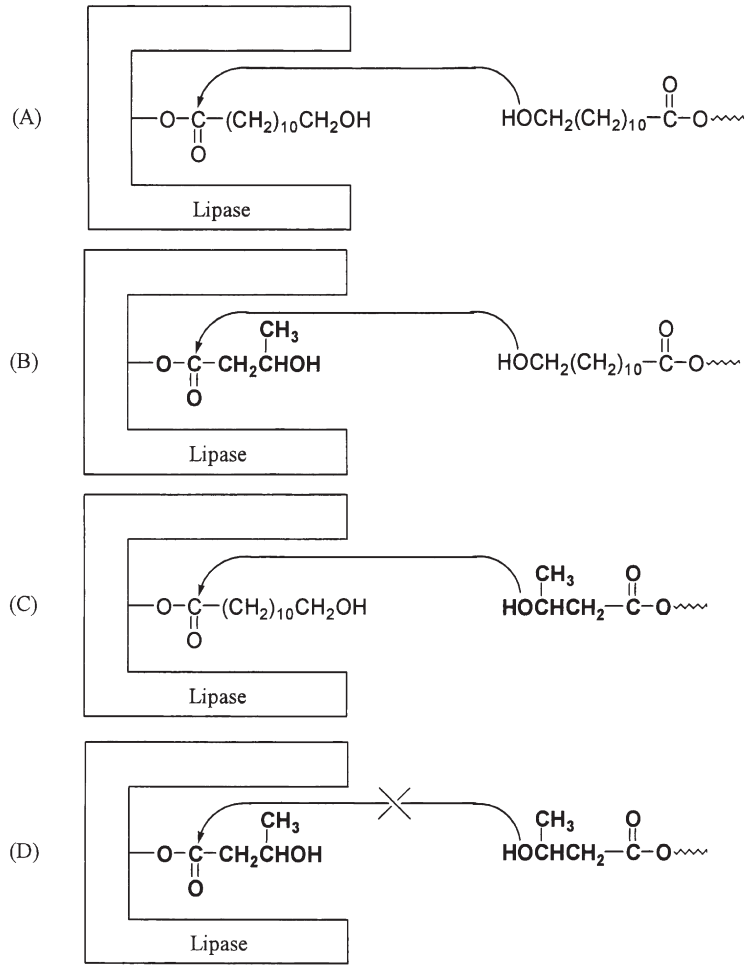


Figure 4.
Four elementary propagations corresponding to the reactions giving A, B, C and D diads.

lection is therefore induced possibly at both acylation and deacylation steps. Thus, the author proposes a new mechanism for the overall ROP reaction with taking the above considerations into account as shown in Figure 5.

In the lipase PF-catalyzed copolymerizations between unsubstituted lactones, typically the copolymerization between PDL and ε-CL produced a copolymer having the diad sequence distribution: PDL-*PDL* = 0.81; PDL-ε-*CL* = 0.19; ε-CL-*PDL* = 0.78; ε-CL-ε-*CL* = 0.22, where the italics denotes the monomer unit of propagating end.[14]

The results show that both ε-CL and PDL monomers have a close polymerizability. And, with the same catalyst the copolymerization of ε-CL with δ-VL gave a similar result.[2] These data suggest that in both copolymerizations the formation of an acyl-enzyme intermediate mainly governs the overall reaction; the effect of a deacylation step is small. This is probably because the structure of propagating ends is very similar in the elementary propaga-

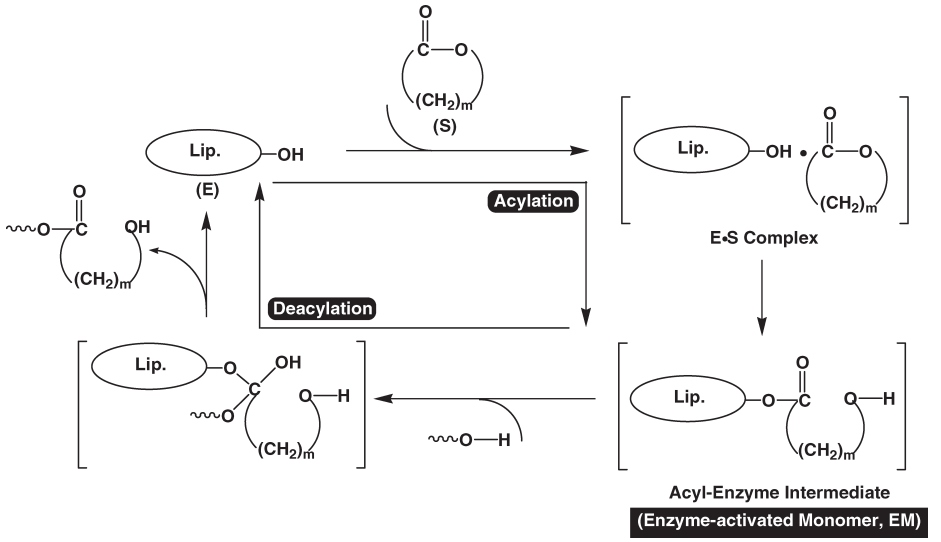


Figure 5.
Proposed mechanism for the overall ROP reaction of lactones with lipase catalyst involving the acylation step and/or the deacylation step as the rate-determining.

tions; all of propagating ends are of primary alcohol structure and the deacylation step is comparable in rate for the copolymerization.

Conclusion

The mechanism of the lipase-catalyzed ROP of lactones has been discussed. Characteristics of the lipase catalysis is, in contrast to the anionic catalysis, the higher reactivity of maclorides (larger ring-sized) than the medium ring-sized lactones like ε-CL, which was explained by the higher rate of an acyl-enzyme intermediate formation for macrolides. A new mechanism of the ROP is proposed, that involves the formation of the acyl-enzyme intermediate (acylation) and/or the nucleophilic attack of the propagating chain end of alcohol on the carbonyl carbon of the intermediate to open the monomer ring (deacylation) as the rate-determining step.

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