NOVEL RING-OPENING POLYMERIZATION OF LACTIDE BY LIPASE

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ABSTRACT

Six-membered D,L-, L,L- and D,D-lactides were polymerized by lipase over a temperature range of 80 to 130 °C to yield the polylactide with a molecular weight (M_W) of greater than 270000. Among the lipases tested, lipase PS gave the greatest molecular weight of polylactide. The polymerization of D,L-lactide by lipase was better than that of L,L- and D,D-lactides. The polymerization of lactide by lipase showed the characteristic features, such as induction period for the initiation of polymerization, formation of oligomer and subsequent formation of high molecular weight polylactide, which may imply the characteristic polymerization by lipase. Immobilization of lipase on celite significantly enhanced the polymerization of lactide particularly with respect to the low concentration of the enzyme and the M_W of the resultant polymer. It was found that there is no clear relationship between enzymatic polymerizability and enzymatic degradability with respect to the enzyme origin and the stereochemistry of lactide.

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INTRODUCTION

Enzymatic polymerization may allow macromolecular architecture for biodegradable and biocompatible materials as it relates to pure and well-defined chemical structures, which is indispensable for both enzymatic degradation and biocompatibility [1]. Above all, lipase-catalyzed polymerization may be one of the most attractive applications in the industrial field for the next generation, because enzymatically prepared polyesters and polycarbonates are expected to be potentially biodegradable. In recent years, enzymatic polymerization using lipase has been studied [2-4], such as polyesters by condensation [5-11], by transesterification [12, 13], or ring-opening polymerizations [14-24], and polycarbonates by ring-opening polymerization of cyclic carbonates [25-27]. However, the history of enzymatic polymerization is not very long and extensive study is needed now.

Polylactide is interesting as a biodegradable and bioabsorbable material. It is produced from lactic acid, either by the direct polycondensation of lactic acid or via the ring-opening polymerization of lactide [28-30]. It is reported that the most effective way to prepare polylactide is by the ring-opening polymerization by anionic, cationic, and coordination initiators [31-39]. However, conventional chemical polymerizations of lactic acid or lactide require extremely pure monomers and anhydrous conditions, as well as metallic catalysts, which must be completely removed before use, particularly for medical applications. To avoid these difficult restrictions for the polymerizations of lactic acid or lactide by the chemical methods, enzyme-catalyzed polymerization may be one of the feasible methods to obtain the polyesters. However, the enzymatic polymerization of lactic acid or lactide has not been reported, except in our communications [23].

In this report, the polymerization of D,L-, L,L- and D,D-lactides using lipase was studied with respect to the polymerization conditions, polymerization profiles and the origin of the enzyme. Also, enzymatic polymerizability and enzymatic degradability were evaluated.

EXPERIMENTAL PROCEDURES

Materials and measurements

D,L-Lactide, D,D-lactide and L,L-lactide were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Aldrich Chemical Co. (Milwaukee, WI, USA), respectively, and used as purchased. Porcine pancreatic lipase (41 U/mg protain, according to the supplier) and lipase from *Candida cylindracea* (500 U/mg, according to the supplier) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Lipase PS was kindly supplied by Amano Pharmaceutical Co., Ltd. (Nagoya, Japan). Novozyme 435 (triacylglycerol hydrolase + carboxylesterase) having 7000 PLU/g (propyl laurate units) was kindly supplied by Novo Nordisk A/S (Bagsvaerd, Denmark). Proteinase K (Tritirachium alkaline proteinase) (20 U/mg, according to the supplier) was purchsed from Wako Chemical Industries, Ltd. (Osaka, Japan). The enzymes were used without further purification. The other materials used were of the highest available purity.

The number-average molecular weight (M_n), weight-averge molecular weight (M_w) and molecular weight dispersion (M_w/M_n) were measured by a gel-permeation chromatograpy (GPC) using GPC columns (Shodex K-803L + K-806L + K-800D, Showa Denko Co., Ltd., Tokyo, Japan) with reflactive index detector. Chloroform was used as the eluent at flow rate of 1.0 mL/min. The GPC system was calibrated with the polystyrene standards. ^{1}H NMR spectra were recorded with a JEOL Model GSX-270 (270 MHz) spectrometer (JEOL Ltd., Tokyo, Japan). ^{13}C NMR spectra were recorded with a JEOL model GSX-270 Fourier Transform Spectrometer operating at 67.5 MHz with complete proton decoupling. Infrared (IR) spectra were measured using a JASCO Fourier Transform Spectrometer model FT/IR-5000 (JASCO Ltd., Tokyo, Japan).

Conversion of lactide to the oligo- and polylactides was determined by comparison of the ^{1}H NMR spectral integration intensities of the δ = 5.0 ppm peak

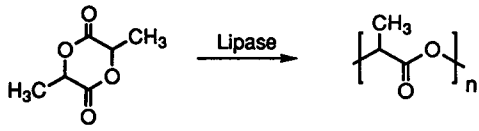
corresponding to the methyne group of the monomeric lactide with the methyne proton of the oligo- and polylactides at $\delta = 5.2$ ppm.

Preparation of polylactide

The polymerization of lactide using commercially available lipases was carried out as shown in Scheme 1. A typical polymerization of poly(D,L-lactide) with a M_w of 69000 (entry 10 in Tab. 1) was carried out as follows: A mixture of lipase PS (15 mg) and D,L-lactide (500 mg) was reacted in an argon atmosphere in a sealed tube placed in a thermostated oil bath at 100 °C for 7 days. After the reaction, the reaction mixture was dissolved in chloroform (5 mL), and the insoluble enzyme was removed by filtration. The chloroform was then evaporated under reduced pressure to quantitatively give the crude polymer. The monomer conversion as determined by ^{1}H NMR was 82%. The crude polymer was further purified by reprecipitation (chloroform as a good solvent; methanol as a poor solvent) to remove any low molecular weight oligomeric fractions to yield the poly(D,L-lactide). The molecular weight and the molecular weight dispersion of the polymer fraction as measured by GPC were M_w = 69000 and M_w/M_n = 1.2, respectively. The spectral data and elemental analysis of poly(D,L-lactide) having an M_w of 69000 are shown as being representative. IR(KBr): 2998, 2950, 1385 (CH), 1757 (ester C=O) cm^{-1}. ^{1}H-NMR (270 MHz: $CDCl_3$): δ = 1.6 (m; CH_3), 5.2 (m; CH). ^{13}C-NMR (67.5 MHz: $CDCl_3$): δ = 16.7 (CH_3), 69.0 (CH), 169.1-169.6 (C=O). Elemental analysis, Found: C, 49.82; H, 5.60%. Calculated for $(C_3H_4O_2)_n$ $(72.06)_n$: C, 50.00; H, 5.60%

Enzymatic degradation by lipase

The enzymatic degradation of polylactide was carried out at 37 °C in 0.1 M potassium phosphate buffer (pH 7.2). Poly(D,L-lactide) having M_w=22000 and M_w/M_n=1.9, poly(L,L-lactide) having M_w=5100 and M_w/M_n=2.3, and poly(D,D-lactide) having M_w=5300 and M_w/M_n=2.2 which were prepared using



Scheme 1

Table 1. Typical ring-opening polymerization of lactide with/without lipase[a)]

Entry[b)]	Lactide	Lipase[c)]	Concentration of lipase based on monomer (%)	Temp. (°C)	Monomer conversion (%)[c)]	Polymer yield based on monomer (%)[d)]	$\overline{M}_w$ [e)]	$\overline{M}_w/\overline{M}_n$
1	D,L-	PPL	5	60	0	0	----	---
2	D,L-	CC	5	60	0	0	----	---
3	D,L-	PS	5	60	0	0	----	---
4	D,L-	Novo	3	80	0	0	----	---
5	D,L-	PPL	3	80	37	29	7500	1,2
6	D,L-	CC	3	80	30	19	8300	1,2
7	D,L-	Pro K	3	80	40	40[f)]	1900	1,2
8	D,L-	PS	3	80	7	3	27000	1,2
9	D,L-	PS	10	80	83	10	36000	1,2
10	D,L-	PS	3	100	82	8	69000	1,2
11	D,L-	PS	3	130	100	16	270000	1,1
12	L,L-	PS	3	80	77	10	15000	1,3
13	L,L-	PS	10	80	86	10	37000	1,1
14	L,L-	PS	3	100	82	8	48000	1,2
15	D,D-	PS	3	80	44	5	13000	1,3
16	D,D-	PS	10	80	73	7	25000	1,2
17	D,D-	PS	3	100	96	5	59000	1,2
18	D,L-	---	--	80	0	0	----	---
19	D,L-	---	--	100	0	0	----	---
20	D,L-	---	--	130	100	100[f)]	3300	1,6
21	L,L-	---	--	100	0	0	----	---
22	D,D-	---	--	100	0	0	----	---

a) Lactide was polymerized in bulk for 7 d.

b) Entries 18-22 : blank tests.

c) Novo: Novozyme 435, PPL: porcine pancreatic lipase, CC: *Candida cylindracea* lipase, Pro K: Proteinase K, PS: Lipase PS.

d) Monomer conversion as determined by the ^{1}H NMR of the reaction mixture.

e) $\overline{M}_w$ of high-molecular weight polymeric fractions of polylactide.

f) Oligomeric fraction was only produced.

lipase by the ring-opening polymerization of D,L-lactide, L,L-lactide and D,D-lactide, respectively, were used in this test. A small thin plate (2.0 mg) of polylactide having the same size for each test was placed in a small glass tube containing 2.0 mL of 0.1 M potassium phosphate buffer. The reaction was started by the addition of 2.0 mg lipase with shaking for 24 h. Biodegradability was evaluated by measuring the dissolved organic compounds in the supernatant of the enzymatic degradation mixture as total organic carbon (TOC) formation using a commercial TOC analyzer. At the same time 0.1 M potassium phosphate buffer containing lipase without the polylactide sample was incubated under the same conditions as a reference test, and the enzyme-derived TOC value was subtracted.

RESULTS AND DISCUSSION

It was found that D,L-, L,L- and D,D-lactides were all polymerized both in bulk and in organic solvents by lipase over a temperature range of 80 to 130 °C to yield the polylactide with a M_W of greater than 270000. Table 1 shows the typical ring-opening polymerization of the lactide with/without lipase. The M_W in Table 1 shows the M_W of polylactide after purification by reprecipitation using chloroform as a good solvent and methanol as a poor solvent. It was confirmed that the lactide was unchanged without lipase at 80 and 100 °C after 7 days, indicating that the lipase catalyzed the polymerization of lactide below 100 °C. After a 7-day incubation of lactide at 130 °C without lipase, the lactide monomer was exclusively converted to the low molecular weight oligomeric fraction with a M_W around 3000. In this case, no high molecular weight polymeric fraction was detected by GPC. On the other hand, D,L-lactide was polymerized at 130 °C in the presence of 3% lipase PS to yield high molecular weight poly(D,L-lactide) with a M_W of 270000 after a 7-day incubation. This indicates that the lipase significantly promoted the polymerization even at 130 °C. On the other hand, at 60 °C lactide was not polymerized by porcine pancreatic lipase, *Candida cylindracea* lipase or lipase PS after a 7-day incubation.

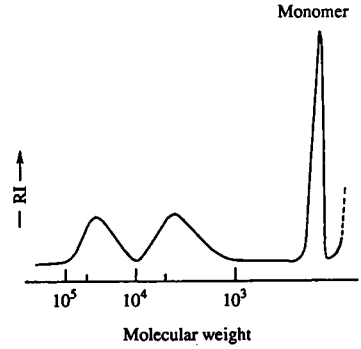


FIG. 1. Gel-permeation chromatography (GPC) profiles of enzymatic polymerization of D,L-lactide using 5% porcine pancreatic lipase at 80 °C for 5 d.

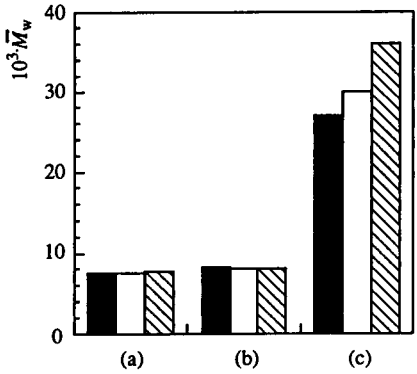


FIG. 2. The molecular weight ($\bar{M}_w$) of the high molecular weight fraction of poly(D,L-lactide) produced by the bulk polymerization of D,L-lactide using 3, 5 and 10% lipase at 80 °C for 7 d. (a) : Porcine pancreatic lipase, (b) : *Candida cylindracea* lipase, (c) : lipase PS

■ : 3% lipase, □ : 5% lipase, ▨ : 10% lipase

Figure 1 shows typical GPC profiles of the enzymatic polymerization of D,L-lactide using 5% PPL at 80 °C after a 5-day incubation. It was observed that the peak area of the monomeric lactide was gradually decreased with the formation of a low molecular weight oligomer peak having a M_W of 1000-3000. Higher molecular weight polymer fraction was then gradually produced, increasing its molecular weight; however, the M_W of the oligomeric fraction remained about the same at around 3000. This phenomenon was well seen in Figure 4 as a typical example. The low molecular weight oligomeric fraction was readily removed by the reprecipitation procedure using chloroform as a good solvent and methanol as a poor solvent. With the addition of methanol, only the low-molecular weight fraction having a M_W of 1200 and a methyl ester group at the terminal was detected according to ^{1}H NMR analysis. With the addition of an excess amount of water, only the dimer and linear tetramer of lactic acid were detected by GPC and ^{1}H NMR analysis, and the monomeric lactic acid, trimer or cyclic oligomer of lactic acid was not produced, suggesting that the polymerization of the lactide by lipase may have occurred due to a diblock-type polymerization. Further analysis for determining the polymerization mechanism is now under way.

In organic solvents, such as 1,4-dioxane and toluene, D,L-lactide was polymerized by lipase to yield oligomeric lactide having a M_W of 610 to 4400 after a 7-day incubation at 80 °C as shown in Table 2. However, the M_W was lower than that obtained by bulk polymerization. It was also observed that both the M_W of polylactide and the monomer conversion were varied according to the solvent used. In an organic solvent, porcine pancreatic lipase gave the greatest molecular weight of polylactide.

It was also found that polymerization occurred with all lipases tested, except for Novozyme 435. However, a significant difference between the enzymes was observed with respect to monomer conversion and the molecular weight of the polymer as shown in Table 1 and Figure 2. Among the lipases tested, lipase PS showed the highest catalytic activities for the polymerization of the lactide with

Table 2. Ring-opening polymerization of D,L-lactide in organic solvent by lipase[a)]

Entry	Lipase[b)]	Solvent	Monomer conversion (%)	Polymer yield(%)	$\overline{M}_w$	$\overline{M}_w/\overline{M}_n$
1	PS	1,4-dioxane	14	14	930	1.2
2	CC	1,4-dioxane	11	11	610	1.2
3	PPL	1,4-dioxane	18	18	1200	1.2
4	PS	toluene	34	34	1700	1.2
5	CC	toluene	28	9	2900	1.1
6	PPL	toluene	44	13	4400	1.1

a) D,L-Lactide was polymerized in 50% solution using 5% lipase at 80°C for 7d.
b) PS: Lipase PS, CC: *Candida cylindracea*, PPL: porcine pancreatic lipase.

Table 3. Ring-opening polymerization of lactide by immobilized lipase on celite[a)]

Entry	Lactide	Lipase[b)]	Concentration of lipase based on monomer (%)	Monomer conversion (%)[c)]	$\overline{M}_w$ [d)]	$\overline{M}_w/\overline{M}_n$
1	D,D-	IM-PS	0.24	>99	23000	4.1
2	L,L-	IM-PS	0.24	>99	26000	4.0
3	D,L-	IM-PS	0.24	>99	29000	4.5
4	D,D-	PS	5	95	11000	2.2
5	L,L-	PS	5	96	8900	2.9
6	D,L-	PS	5	96	34000	13.8

a) Lactide was polymerized in bulk at 100°C for 7d.
b) PS: Lipase PS, IM-PS: Immobilized lipase PS on celite.
c) Monomer conversion as determined by the ^{1}H NMR of the reaction mixture.
d) $\overline{M}_w$ of entire polymeric and oligomeric fractions of lactide produced by the conversion of lactide.

respect to M_W as indicated in Figure 2. Therefore, further studies on the polymerization of lactide were carried out using lipase PS.

Polylactide produced by the lipase-catalyzed polymerization of lactide in bulk showed two peaks in GPC as shown in Figure 1. This is characteristic of the lipase-catalyzed polymerization of lactide in bulk, which may be ascribed to the polymerization mechanism of lactide by lipase. In order to evaluate the homogeneity of lipase used in this test, lipases were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) by the usual method. As a result lipases used in these tests, except for PPL, gave a single band on SDS-PAGE. Therefore, the two peaks of the polymeric fraction on GPC may not be caused by contaminated enzymes. For further analysis of the enzymatic polymerization of lactide, the time course of the polymerization of D,L-lactide in bulk using lipase was measured. Figure 3 shows the time course of the M_W change of the entire polymeric and oligomeric fractions and the monomer conversion in the bulk polymerization of lactide using lipase PS at 80 °C. Figure 4 shows the time course of the M_W change for high molecular weight polylactide and low molecular weight oligomeric lactide, respectively, for the D,L-lactide polymerization in bulk by lipase PS at 80 °C. It was found that the polymerization of lactide occurred after an induction period of about 1 day for 10% lipase concentration and about 3 days for 5% and 3% lipase concentrations. These induction periods may be related to the alteration (activation) of lipase in favor of the polymerization of lactide to its oligomer. It was also observed that the conversion rate was increased after about 5 days for 10% lipase concentration and 7 days for 3% lipase concentration. After this time, the M_W of the higher molecular weight fraction was more quickly increased compared to that of the oligomeric fraction. This phenomenon may be due to further alteration (activation) of the lipase to favor the polymerization towards the formation of higher molecular weight polylactide. Similar tendencies were observed for lactide polymerization at 100 °C.

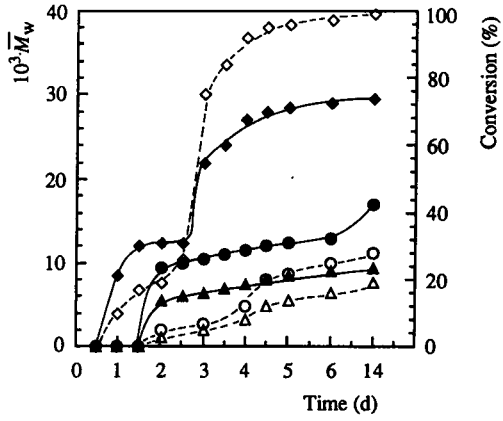


FIG. 3. Time course of $\overline{M}_w$ change in the entire polymeric and oligomeric fractions and the conversion in the D,L-lactide polymerization in bulk using lipase PS at 80 °C.
$\overline{M}_w$; ◆ : 10%, ● : 5%, ▲ : 3% lipase
Conversion; ◇ : 10%, ○ : 5%, △ : 3% lipase

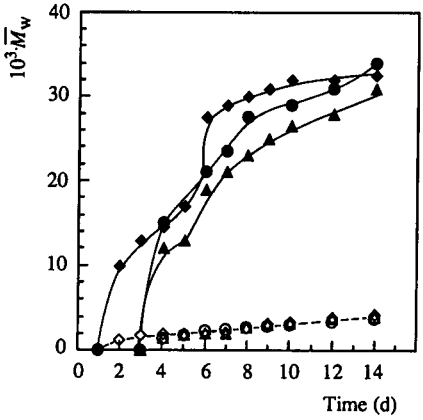


FIG. 4. Time course of $\overline{M}_w$ change for high molecular weight polylactide and low molecular weight oligomeric lactide in the bulk polymerization using lipase PS at 80 °C (Conditions were the same as those of FIG. 3).
High molecular weight $\overline{M}_w$; ◆ : 10%, ● : 5%, ▲ : 3% lipase
Low molecular weight $\overline{M}_w$; ◇ : 10%, ○ : 5%, △ : 3% lipase

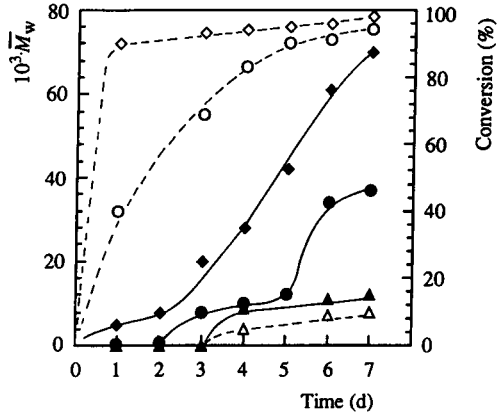


FIG. 5. Time course of $\overline{M}_w$ change of the entire polymeric and oligomeric fractions and the conversion in the D,L-lactide polymerization in bulk using 5% lipase PS.
$\overline{M}_w$; ◆ : 130 °C, ● : 100 °C, ▲ : 80 °C
Conversion; ◇ : 130 °C, ○ : 100 °C, △ : 80 °C

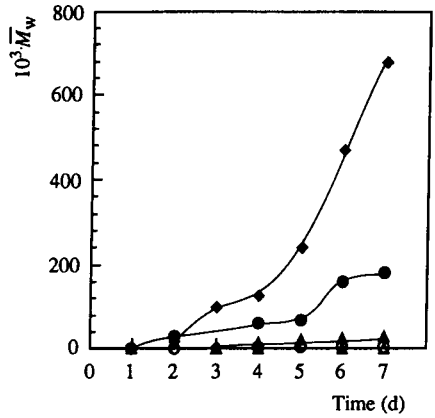


FIG. 6. Time course of $\overline{M}_w$ change for high molecular weight polylactide and low molecular weight oligomeric D,L-lactide in the bulk polymerization using 5% lipase PS.
High-molecular weight $\overline{M}_w$; ◆ : 130 °C, ● : 100 °C, ▲ : 80 °C
Low-molecular weight $\overline{M}_w$; ◇ : 130 °C, ○ : 100 °C, △ : 80 °C

Also, similar tendencies were obsrved for lactide polymerization by *Candida cylindracea* lipase and porcine pancreatic lipase. For lactide polymerization by *Candida cylindracea* lipase and porcine pancreatic lipase, the induction time was shorter than that by lipase PS. However, the M_W of the resultant polymer was greater when lipase PS was used. In summary, the enzyme concentration in the polymerization of lactide affected the rate of monomer conversion and molecular weight increase. However, the M_W of the polymeric fraction was almost the same depending on the polymerization time. Almost the same M_W was obtained regardless of enzyme concentration. The M_W of polylactide was also dependent on the lipase origin.

The polymerization temperature significantly influenced the monomer conversion and the M_W of the resultant polylactide. Figures 5 and 6 show the time course of the bulk polymerization of D,L-lactide by 5% lipase PS. The conversion rate and the molecular weight of the polylactide were significantly increased with increasing reaction temperature from 80 to 130 °C. It has been observed that D,L-lactide was thermally polymerized at 130 °C without lipase to produce a M_W of 3000; however, the M_W of polylactide was much greater in the presence of lipase. By the addition of 5% lipase PS, a M_W of 700000 was attained at 130 °C, indicating that the lipase actually promoted the polymerization of lactide even at 130 °C. The induction period for the polymerization of lactide was observed at 80 and 100 °C, but was not clear at 130 °C.

Figures 7 and 8 show the time course of the polymerization of D,L-, D,D- and L,L-lactides by 5% lipase PS at 100 °C. It was found that similar polymerization curves were obtained during the first 5-day polymerization. However, after 5 days, the M_W of poly(D,L-lactide) was quickly increased to 180000, whereas the M_W of poly(D,D-lactide) and poly(L,L-lactide) was slightly increased to 60000 after the 7-day polymerization. This may be due to the polymer conformation or alteration (activation) of the enzyme caused by the monomeric or oligomeric D,L-lactide in

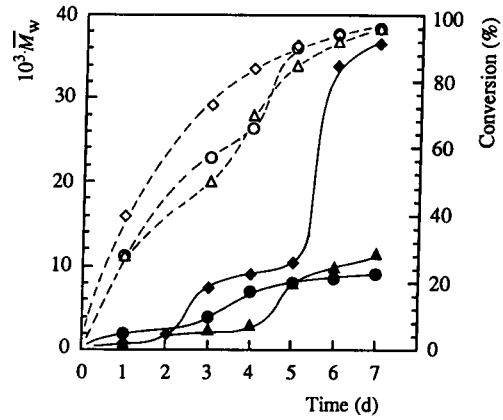


FIG. 7. Time course of $\overline{M}_w$ change of the entire polymeric and oligomeric fractions and the conversion in the bulk polymerization of D,L-, L,L-, and D,D-lactide polymerization in bulk using 5% lipase PS at 100 °C.
$\overline{M}_w$; ◆ : D,L-, ● : L,L-, ▲ : D,D-lactide
Conversion; ◇ : D,L-, ○ : L,L-, △ : D,D-lactide

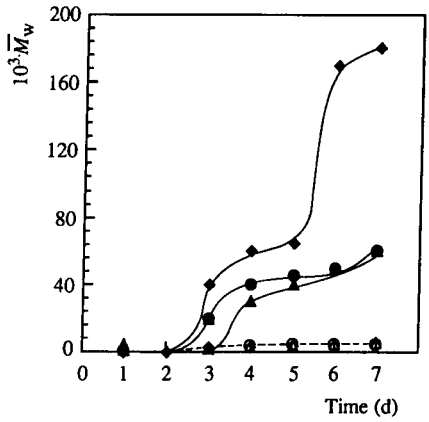


FIG. 8. Time course of $\overline{M}_w$ change for high-molecular weight polylactide and low-molecular weight oligomeric lactide in the bulk polymerization using 5% lipase PS at 100 °C.
High molecular weight $\overline{M}_w$; ◆ : D,L-, ● : L,L-, ▲ : D,D-lactide
Low molecular weight $\overline{M}_w$; ◇ : D,L-, ○ : L,L-, △ : D,D-lactide

favor of the formation of high molecular weight polymers. Similar tendencies were observed for porcine pancreatic lipase and *Candida cylindracea* lipase.

The specific rotation, defined as $[\alpha]_D^{25} = \alpha/(\gamma \cdot l)$ where γ is the mass concentration in the solvent and l is the light path length, was measured in chloroform at a concentration of 10 g/L at 25 °C at a wavelength of 589 nm. No significant enantioselection in the lipase-catalyzed polymerization of D,L-lactide was observed by lipase PS. That is, the specific rotation of poly(D,L-lactide) was $[\alpha]_D^{25}$ = +9.4 ° (c=1.0, $CHCl_3$) (entry 10 in Tab. 1). However, optically active poly(L,L-lactide) was obtained from L,L-lactide using lipase PS. The specific rotation for poly(L,L-lactide) was $[\alpha]_D^{25}$ = +141° (c=1.0, $CHCl_3$) (entry 12 in Tab. 1). This specific rotation was in agreement with that of the literature of $[\alpha]_D^{25}$ = +153° (c=1.0, $CHCl_3$) [24].

Table 3 shows the polymerization results by immobilized lipase PS on celite and by naked lipase PS at 100 °C. The immobilization of lipase PS on celite significantly enhanced the polymerization of lactide particularly with respect to the low enzyme concentration and the molecular weight of the resultant polymer. That is, 5% immobilized lipase PS, corresponding to 0.24% enzyme concentration, practically catalyzed the polymerization of lactide to yield the polylactide an over 99% monomer conversion. The immobilization effect was clearly apparent for D,D- and L,L-lactides, and the molecular weight of the resultant polymer was over two fold of those obtained using 5% naked lipase PS at 100 °C for 7 days. The immobilization of lipase on celite may cause a favorable alteration at the active site of the lipase for polymerization, in addition to the stabilization of the enzyme and the dispersibility in the bulk polymerization of the lactide. Further studies are now under way.

Enzymatic degradability was evaluated using lipases from various origins in a potassium phosphate buffer (pH 7.2) at 37 °C for 24 h. The results are shown in Figure 9. It was found that the polylactides were degraded by lipase with a significant difference from non-enzymatic hydrolysis. Enzymatic degradability was

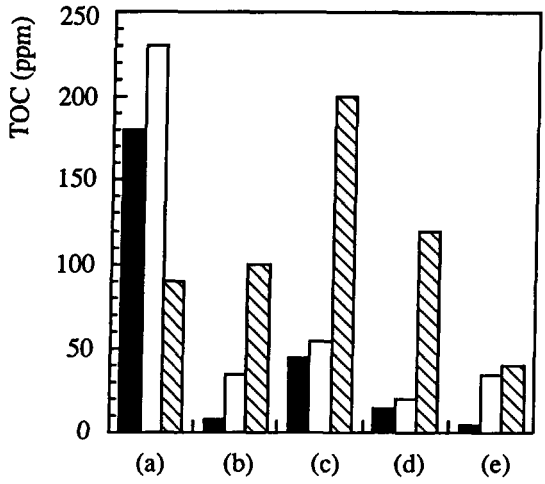


FIG. 9. Total organic carbon (TOC) concentration by the enzymatic degradation of polylactide in 0.1 M potassium phosphate buffer (pH 7.2) at 37 °C for 24 h.
(a): Proteinase K, (b): Lipase PS, (c): Porcine pancreatic lipase, (d): *Candida cylindracea* lipase, (e): Blank
■ : poly(D,L-lactide) with $\overline{M}_w$=22000 and $\overline{M}_w/\overline{M}_n$=1.9; □ ; poly(L,L-lactide) with $\overline{M}_w$=5100 and $\overline{M}_w/\overline{M}_n$=2.3, ▨ : poly(D,D-lactide) with $\overline{M}_w$=5300 and $\overline{M}_w/\overline{M}_n$=2.2.

varied according to the origin of the enzyme as well as the chirality of the lactide. Poly(D,L-lactide) and poly(L,L-lactide) were readily degraded by proteinase K. However, the degradability of poly(D,D-lactide) by proteinase K was considerably decreased. Poly(D,D-lactide) was degraded by porcine pancreatic lipase and *Candida cylindracea* lipase. It was found that there was no clear relationship between enzymatic degradability and enzymatic polymerizability with respect to the enzyme origin.

In conclusion, it was found that a six-membered lactide was polymerized by lipase over a temperature range of 80 to 130 °C to yield polylactides with a M_W of greater than 270000. Among the lipases tested, lipase PS showed the best results with respect to both the polymerization rate and the molecular weight of the resultant polylactide. Also, the polymerization results of the D,L-lactide were better than those of the D,D- and L,L-lactides. Some characteristic polymerization profile of lactide by lipase was confirmed, which implies the polymerization mechanism by lipase. Further studies are needed now. The immobilization of lipase on celite significantly enhanced the polymerization of lactide with respect to the low concentration of the enzyme and the M_W of the resultant polymer. There is no clear relationship between enzymatic polymerizability and enzymatic degradability with respect to the enzyme origin.

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