Enzyme-Catalyzed Ring-Opening Polymerization of Cyclic Esters in the Presence of Poly(ethylene glycol)

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ABSTRACT: Low-molecular-weight polyesters were obtained by the ring-opening homopolymerization and copolymerization reaction of ε-caprolactone, *rac*- or L-lactide, and glycolide in the presence of poly(ethylene glycol) with an enzyme catalyst, a lipase from *Candida antarctica, Pseudomonas cepacia*, or *Pseudomonas fluorescens*. The influence of several parameters, including time, temperature, and enzyme and monomer concentration, on the polymerization rate was studied. The resulting polymers were characterized by ^{1}H-NMR or ^{13}C-NMR, Fourier transform infrared spectroscopy, matrix-assisted laser desorption/ionization–time of flight mass spectroscopy, and gel permeation chromatography. J Appl Polym Sci 000: 000–000, 2012

Key words: biodegradable; biomaterials; enzymes; polyesters; ring-opening polymerization

INTRODUCTION

Aliphatic polyesters are widely used for medical applications, such as drug-delivery systems or resorbable implants.[1–9] Poly(ε-caprolactone) (PCL), polyglycolide, polylactide (PLA), and their copolymers are degraded by the hydrolysis of their ester linkages in physiological conditions, such as in the human body. The degradation kinetics of polyesters can be tailored by the modification of their morphologies and hydrophilicities. There are two methods for preparing polyesters: polycondensation and ring-opening polymerization (ROP). ROP gives polyesters with a higher molecular weight and a lower polydispersity (PD), so it is the preferred route. The ROP of cyclic esters can be led in the presence of anionic or cationic initiators, a metal coordinate, and enzymatic catalysts.[10,11] Enzymatic ring-opening polymerization (e-ROP) has many advantages. Enzymes are chemoselective, regioselective, stereoselective, and enantioselective; with them, one can replace expensive, difficult to use, and toxic catalysts.[12] The enzymes used are nontoxic to the environment and easily biodegradable. e-ROP has been studied very extensively. A wide number of enzymes from different origins has been tested in organic synthesis, including lipases from *Candida cylindracea* (CcLipase), *Pseudomonas fluorescens* (PfLipase), *Porcine pancreas* (PpLipase), *Aspergillus niger*, *Candida rugosa*, *Penicillium roqueforti*, *Pseudomonas cepacia* (PcLipase), *Rhizopus japanicus*, *Pseudomonas* sp. (PsLipase), *Candida antarctica* (CaLipase), *Pseudomonas aeroginosa* (PaLipase), and *Rhyzopus delemer*.[13–26]

E-caprolactone (CL) is the most studied lactone in the lipase-catalyzed ROP method. PCL, with a molecular weight ranging from 540 to 17,800 g/mol, has been synthesized under various polymerization conditions. The temperature and time of e-ROP have been varied from 20 to 60°C and from 1 h to 20 days, respectively. The bulk e-ROP process enables one to obtain a PCL having a number-average molecular weight (M_n) in the range 1100–12,000 g/mol. However, the e-ROP of CL in a medium [toluene, 1-butyl-3-methylimidazolium hexafluorophosphate, 1-butyl-3-methylimidazolium tetrafluoroborate, or 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide] has resulted in a polymer with an M_n of 540–17,800 g/mol. For CaLipase, PcLipase, and PfLipase, the corresponding CL yields have been 44–100, 84, and 71–99%, respectively.[1,14,16,17,24]

e-ROP of *rac*-lactide (*rac*-LA) or L-lactide (LLA) has been performed with PsLipase, immobilized PsLipase on celite, PpLipase, *Tritirachium alkaline* proteinase, and CcLipase at 60–100°C for 3–7 days. The M_n of PLA varied from 1200 to 59,000 g/mol, and the monomer conversion was 18–99%.[1,14]

Mechanisms for e-ROP using lipases have been proposed by several authors. First, the lipase reacts with the monomer to form a lipase-activated CL

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complex; then, the alcohol reacts with the complex.[13,24,25] Dong et al.[27] postulated a new mechanism. They showed that the water content in the reaction medium is extremely important.

The e-ROP of cyclic esters has been studied many times. However, the ring-opening homopolymerization and copolymerization of CL, LLA, *rac*-LA, and glycolide (GL) using CaLipase, PcLipase, PfLipase, and poly(ethylene glycol) (PEG) has not been until now. In this study, oligoester diols were prepared by the lipase-catalyzed ring-opening homopolymerization or copolymerization of LLA, *rac*-LA, CL, and GL. The effects of temperature, reaction time, and lipase or PEG dosage on the ROP process were examined.

EXPERIMENTAL

Materials

GL (1,4-dioxane-2,5-dione, 99%), CL (2-oxepanone, 99%), and 3,6-dimethyl-1,4-dioxane-2,5-dione (98%, *rac*-LA and LLA) were purchased from Aldrich, Germany. Before use, CL was dried and distilled over CaH_2 at reduced pressure. *rac*-LA and LLA were crystallized from a mixture of dry toluene with hexane and dried at room temperature *in vacuo*. GL, PEG (M_n = 400, Fluka), dichloromethane (pure, POCh Joint-Stock Co., Poland), and methanol (pure, POCh Joint-Stock Co.) were used as received. All lipases (CaLipase, PcLipase, and PfLipase) were used without further purification.

Lipase-catalyzed ROP of CL

All reactions were carried out in bulk. Before the reaction, the monomer (2 g, 17.5 mmol) and lipase (25, 50, or 100 mg) were dried *in vacuo* at room temperature for 1 h. Next, CL, PEG, and the lipase were placed in 10-mL ampules under an inert dry argon atmosphere. The ampules were placed in an oil bath maintained at 60–100°C for predetermined time periods. After the reaction time was complete, the mixture was dissolved in dichloromethane, and the insoluble enzyme was removed by filtration. Next, the obtained solution was washed with methanol under vigorous stirring. The latter operation was repeated three times. The isolated polymer was dried *in vacuo* at room temperature for 72 h.

Lipase-catalyzed ROP of LLA and *rac*-LA

A typical procedure was as follows. The monomers and enzymes were vacuum-dried for 1 h. To a mixture of lactide (2.52 g, 17.5 mmol), PEG, and toluene (5 mL) at 60–80°C, the enzyme (25, 50, or 100 mg) was added under an argon atmosphere. After the reaction, the enzyme was removed by filtration, and the polymer was isolated by precipitation in methanol. The latter operation was repeated three times. The isolated polymer was dried *in vacuo* at room temperature for 72 h.

Lipase-catalyzed ring-opening copolymerization of CL and GL

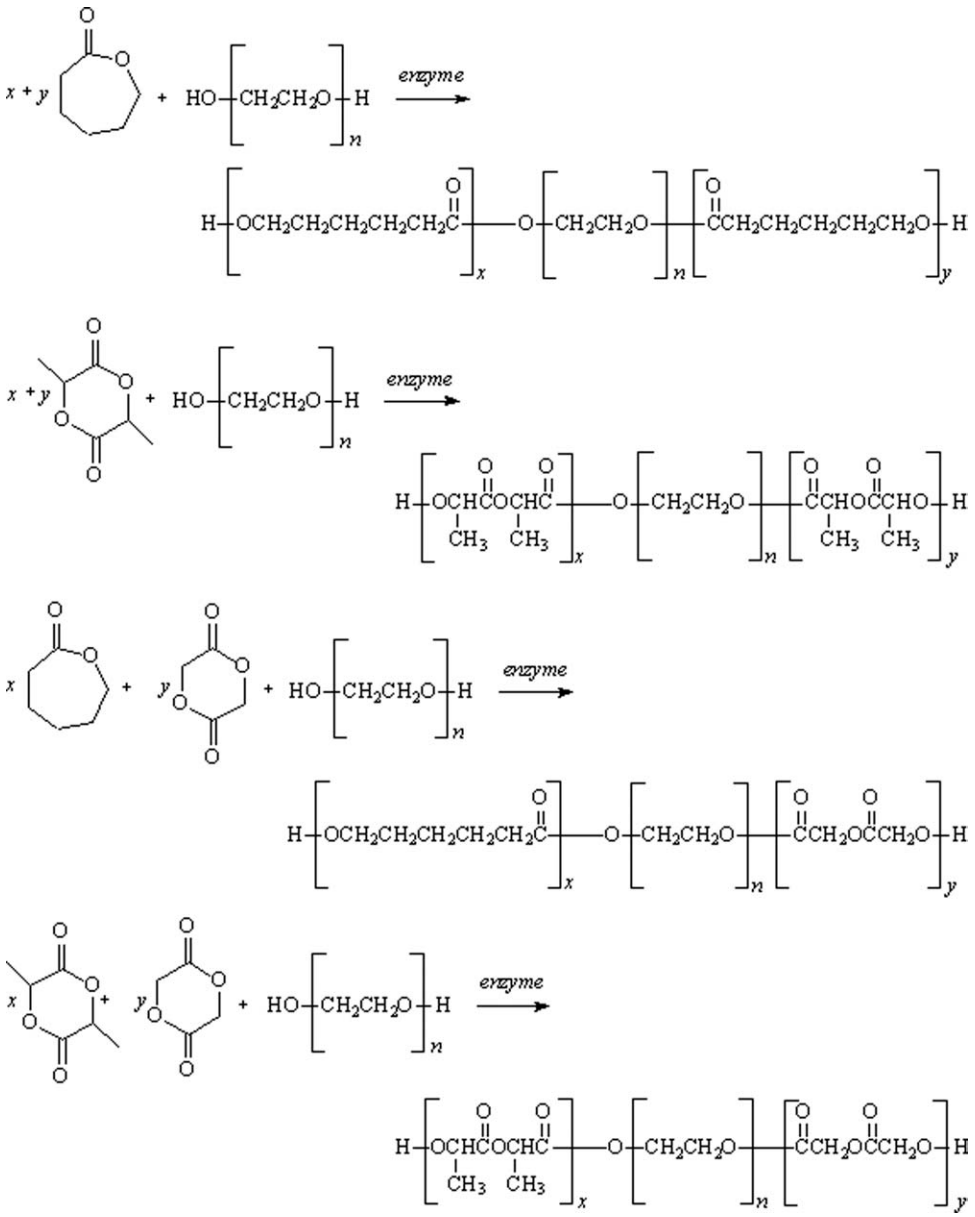
GL and CL were copolymerized in bulk. Before the reaction, CL (1 g, 8.75 mmol), GL (1.02 g, 8.75 mmol), and lipase (50 mg) were dried *in vacuo* at room temperature for 1 h. Next, CL, GL, PEG, and lipase were placed in 10-mL ampules under an inert dry argon atmosphere. The ampules were sealed and heated in a silicone oil bath. After the reaction, the copolymers were dissolved in chloroform, and the enzyme was removed by filtration. Then, the product was precipitated in methanol. The precipitated polymers were dried *in vacuo* at room temperature for 24 h.

Lipase-catalyzed ring-opening copolymerization of *rac*-LA and GL

Typically, the GL, *rac*-LA, and enzymes were vacuum-dried for 1 h. To a mixture of GL (1.02 g, 8.75 mmol), *rac*-LA (1.26 g, 8.75 mmol), PEG, and toluene (2 mL), enzyme (50 mg) was added under a dry argon atmosphere. The ampules were placed in an oil bath maintained at 60–100°C for 14 days. After the reaction, the enzyme was removed by filtration, and the cooled crude products were dissolved in chloroform and precipitated with methanol. These precipitates were dried *in vacuo* at room temperature for 24 h.

Measurements

The ^{1}H-NMR and ^{13}C-NMR spectra of the polyesters were obtained by use of a Varian 300-MHz spectrometer with $CDCl_3$ as the solvent. The IR spectra were measured from KBr pellets (PerkinElmer spectrometer, Great Britain). The molecular mass values and molecular mass distributions of the polymers were determined at 308 K on a Lab Alliance gel permeation chromatograph equipped with Jordi Gel DVB mixed bed (250 × 10 mm) columns and a refractive detector with tetrahydrofuran or chloroform as the eluent (1 mL/min). The molecular mass scale was calibrated with polystyrene standards. The matrix-assisted laser desorption/ionization (MALDI)–time of flight (TOF) mass spectroscopy (MS) spectra were obtained in the linear mode on a Kompact MALDI 4 Kratos analytical spectrometer with a nitrogen gas laser and 2-[(4-hydroxyphenyl)diazenyl] benzoic acid as the matrix. The polymer viscosity



Scheme 1 Reaction scheme for the synthesis of aliphatic polyesters.

was measured in *N,N*-dimethylformamide (at 30°C) on a Stabinger viscometer (SVM 3000, Österreich).

RESULTS AND DISCUSSION

The aim of this research was to obtain low-molecular-weight polyesters terminated at both sides by hydroxyl groups that could be subsequently used as polyester conjugates of drugs.[7–9] Metal compounds are most often used as catalysts in the ROP of cyclic esters. However, they are not preferred for biomedical applications because of their toxicity. It is known that lipases show catalytic activity for the ROP of cyclic esters.[13–26] The e-ROP of CL, LLA, *rac*-LA, and GL in the presence of PEG was examined. The process was performed in bulk or in toluene. Scheme 1 presents the synthetic procedure that was used in this study.

ROP of cyclic esters

At first, the e-ROP of CL was carried out with a lipase (CaLipase, PcLipase, or PfLipase) as a catalyst at 60–100°C. The polymerizations were carried out in bulk for 7–21 days. The polymerization results are summarized in Table I.

The structure of PEG–PCL was characterized by ^{1}H-NMR or ^{13}C-NMR and IR spectroscopy (Fig. 1). The results clearly indicate that typical ^{1}H-NMR chemical shifts at 1.34 ppm (—$OCH_2CH_2CH_2$—), 1.59 ppm (—$OCH_2CH_2CH_2$—), 2.28 ppm [—$O(O)CCH_2$—], 3.65 ppm (—OCH_2CH_2O—), and 4.03 ppm

TABLE I
Enzyme-Catalyzed ROP of CL in the Bulk

Code	CL/PEG molar ratio	Enzyme (mg)	Time (days)	Temperature (h)	Yield (%)	M_n (Da)[a]	PD[a]	M_n (Da)[b]	PD[b]	η_{inh} (dL/g)[c]
1	25 : 1	CaLipase (50)	14	80	91	2500	1.18	—	—	0.10
2	25 : 1	PcLipase (50)	14	80	78	2200	1.21	—	—	0.08
3	25 : 1	PfLipase (50)	14	80	65	2100	1.23	—	—	0.08
4	50 : 1	CaLipase (25)	14	80	49	2900	1.24	2700	1.19	0.09
5	50 : 1	CaLipase (50)	14	60	32	2100	1.19	—	—	0.07
6	50 : 1	CaLipase (50)	7	80	62	3400	1.31	—	—	0.10
7	50 : 1	CaLipase (50)	14	80	82	4400	1.24	3900	1.28	0.12
8	50 : 1	PcLipase (50)	14	80	67	3900	1.28	2700	1.21	0.11
9	50 : 1	PfLipase (50)	14	80	53	3000	1.20	2300	1.18	0.09
10	50 : 1	CaLipase (50)	21	80	89	4700	1.28	—	—	0.12
11	50 : 1	CaLipase (50)	14	100	86	4600	1.27	—	—	0.12
12	50 : 1	CaLipase (100)	14	80	85	4700	1.30	—	—	0.12
13	100 : 1	CaLipase (50)	14	60	24	2900	1.33	—	—	0.09
14	100 : 1	CaLipase (50)	14	80	48	5200	1.35	4100	1.18	0.13
15	100 : 1	CaLipase (50)	7	80	39	5000	1.32	—	—	0.11

[a] Determined by GPC.
[b] Determined by MALDI–TOF MS.
[c] Measured at 30°C in DMF.

[—C(O)OCH$_2$—] ppm were associated with PEG–PCL. The peaks at 24.1 ppm (—CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$—), 25.1 ppm (—CH$_2$CH$_2$COO—), 27.9 ppm [—CH$_2$CH$_2$OC(O)—], 33.6 ppm (—CH$_2$CH$_2$COO—), 63.7 ppm [—CH$_2$CH$_2$OC(O)—], and 173.1 ppm [—C(O)O—] were observed in all products obtained by the polymerization of CL. The absorption peaks at 1721 and 2943 cm^{-1} were associated with the functional groups C=O and —CH, respectively.

The corresponding CL/PEG molar ratios were 25 : 1, 50 : 1, and 100 : 1. Experiments were conducted with three different levels of lipase concentration at the same scale of CL (25, 50, and 100 mg of CaLipase). Under these conditions, the oligomerization of CL occurred when PEG was used as an initiator and when enzymes were employed as insertion catalysts. CaLipase was significantly more efficient and led to higher values of M_n and yield than PfLipase or PcLipase.

The M_n's determined from gel permeation chromatography (GPC) for PCL were in the 2100–5200-Da range, and the PD indices were in the 1.18–1.35 range. The molecular mass of PCL was dependent on the CL/PEG molar ratio. The trend was that when the amount of initiator was increased, the average M_n of PCL increased. As shown in Table I, PCL products were obtained with M_n's (from GPC) of 2500, 2900, and 5200 Da for **1, 4,** and **14,** respectively. Both the inherent viscosity (η_{inh}) and the molecular mass of PCL increased when the reaction temperature was raised from 60 to 100°C. For **5, 7,** and **11,** the M_n (from GPC) values were 2100, 4400, and 4600 Da, respectively.

It was found that CaLipase gave higher yields than PcLipase or PfLipase. As shown in Table I, for **1, 2,** and **3,** the corresponding CL yields were 91, 78, and 65%. Similarly, the yields of PCL for **7, 8,** and **9** were 82, 67, and 53%, respectively. The yield of PCL increased with the amount of lipase. For **4** (25 mg of CaLipase), **7** (50 mg of CaLipase), and **12** (100 mg of CaLipase), the corresponding yield values were 49, 82, and 85%.

In the MALDI–TOF MS of PCL, linear and cyclic polyesters were detected. The most prominent series of peaks was characterized by a mass increment of 114 Da, which was equal to the mass of the repeating unit in PCL. This series was assigned to PCL terminated with a hydroxyl group and was detected as the Na^+ adduct [residual mass (RM) = 41 Da]. The second series of low-intensity peaks corresponded to

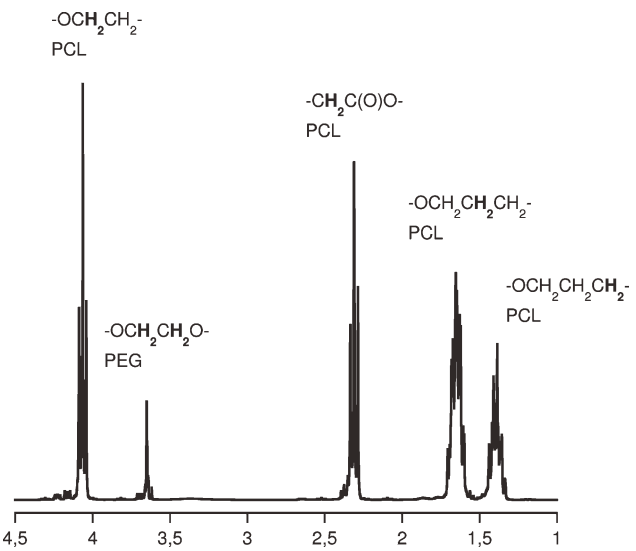


Figure 1 ^{1}H-NMR spectrum of PEG–PCL (in $CDCl_3$).

TABLE II
Enzyme-Catalyzed ROP of LLA and *rac*-LA

Code	LA/PEG molar ratio	Enzyme (mg)	Time (days)	Temperature (h)	Yield (%)	M_n (Da)[a]	PD[a]	M_n (Da)[b]	PD[b]	η_{inh} (dL/g)[c]
16	25 : 1[d]	CaLipase (50)	14	80	79	2900	1.22	—	—	0.11
17	25 : 1[d]	PcLipase (50)	14	80	63	2400	1.27	—	—	0.10
18	25 : 1[d]	PfLipase (50)	14	80	55	2000	1.20	—	—	0.10
19	50 : 1[d]	CaLipase (25)	14	80	42	3200	1.31	2400	1.18	0.11
20	50 : 1[d]	CaLipase (50)	14	60	27	1900	1.17	—	—	0.10
21	50 : 1[d]	CaLipase (50)	7	80	50	3200	1.33	—	—	0.12
22	50 : 1[d]	CaLipase (50)	14	80	65	4200	1.27	3200	1.22	0.14
23	50 : 1[d]	PcLipase (50)	14	80	52	3800	1.23	2900	1.24	0.12
24	50 : 1[d]	PfLipase (50)	14	80	41	3100	1.29	2600	1.21	0.11
25	50 : 1[e]	CaLipase (50)	14	80	62	4200	1.28	—	—	0.14
26	50 : 1[e]	PcLipase (50)	14	80	53	3700	1.21	—	—	0.13
27	50 : 1[e]	PfLipase (50)	14	80	40	2800	1.25	—	—	0.11
28	50 : 1[d]	CaLipase (50)	21	80	67	4600	1.35	—	—	0.15
29	50 : 1[d]	CaLipase (100)	14	80	62	4200	1.41	—	—	0.14
30	100 : 1[d]	CaLipase (50)	14	60	18	2200	1.29	—	—	0.11
31	100 : 1[d]	CaLipase (50)	14	80	34	4700	1.39	—	—	0.14
32	100 : 1[d]	CaLipase (50)	7	80	26	3300	1.37	—	—	0.12

Medium: toluene
[a] Determined by GPC.
[b] Determined by MALDI–TOF.
[c] Measured at 30°C in DMF.
[d] *rac*-LA.
[e] LLA.

cyclic molecules (RM = 23 Da, Na^+ adduct). As shown in Table I, the determined average M_n and PD values were in reasonable agreement for the two techniques (GPC and MALDI–TOF MS).

Next, the ROP of LLA and *rac*-LA was carried out at 60–80°C for 7–21 days in toluene. The process was catalyzed by CaLipase, PfLipase, and PcLipase. The LLA(or *rac*-LA)/PEG molar ratios were 25 : 1, 50 : 1, and 100 : 1. Table II shows the molecular weights and yields of the polymer.

The structure of PEG–PLA was characterized by ^{1}H-NMR and ^{13}C-NMR (Fig. 2). The chemical shifts at 1.62 ppm [—CH(CH_3)—], 3.65 ppm (—OCH_2CH_2O—), and 5.15 ppm [—CH(CH_3)—] were assigned to the methylene protons in PEG–PLA. Peaks at 16.8 ppm (—CH_3), 69.2 ppm [—CH(CH_3)—], and 169.80 ppm [—C(O)O—] were observed in the ^{13}C-NMR spectra. The infrared spectra clearly showed characteristic bands at 2997 cm^{-1} (υas_{CH3}) and 1760 ($\upsilon_{C=O}$) cm^{-1}.

The yields of PLA were about 18–79%. This yield increased with decreasing LLA or *rac*-LA/PEG feed ratio and increasing temperature. For **16, 19,** and **31,** the yield values were 79, 42, and 34%, respectively. Similarly, the yield values for **20** and **22** were 27 and 65%, respectively.

The efficiency values of CaLipase, PcLipase, and PfLipase in the polymerization of *rac*-LA or LLA were analogous, as in the case of the polymerization of CL.

The M_n's determined from GPC for PLA were in the 1900–4700-Da range, and the PD indices were in the 1.17–1.41 range. The average molecular mass values of PLA determined by the MALDI–TOF MS method were in the 2400–3200-Da range. The MALDI–TOF MS spectrum of PLA was composed of two series of peaks. The main series came from PLA terminated with a hydroxyl group and corresponded

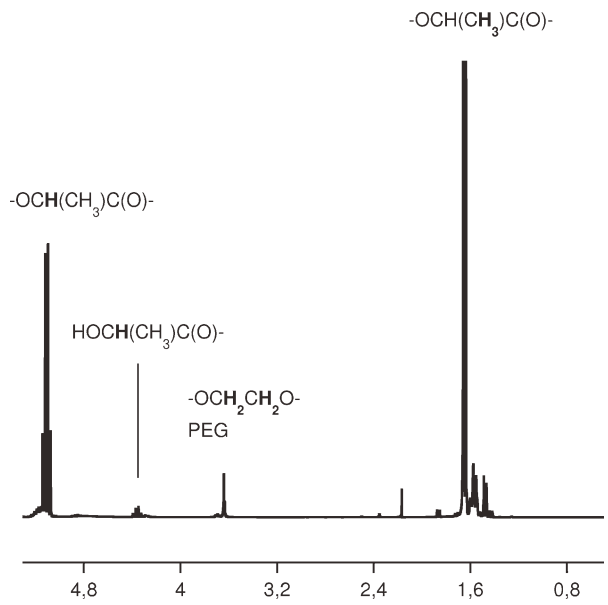


Figure 2 ^{1}H-NMR spectrum of PEG–PLA (in $CDCl_3$).

TABLE III
Enzyme-Catalyzed Ring-Opening Copolymerization of CL, *rac*-LA, and GL

Code	Comonomer	CL (*rac*-LA)/GL/ PEG molar ratio	Enzyme (mg)	Temperature (h)	Yield (%)	M_n (Da)[a]	PD[a]	η_{inh} (dL/g)[b]	L_{CL} ($L_{rac\text{-LA}}$) (% mol)[c]
33	CL/GL	12.5:12.5:1	CaLipase (50)	80	65	2100	1.23	0.09	59
34	CL/GL	12.5:12.5:1	PcLipase (50)	80	56	1900	1.25	0.09	60
35	CL/GL	12.5:12.5:1	PfLipase (50)	80	49	1700	1.18	0.09	69
36	CL/GL	25:25:1	CaLipase (50)	60	25	1800	1.17	0.09	64
37	CL/GL	25:25:1	CaLipase (50)	80	61	3600	1.26	0.12	57
38	CL/GL	25:25:1	CaLipase (50)	100	64	3700	1.29	0.12	62
39	CL/GL	50:50:1	CaLipase (50)	80	36	4400	1.31	0.13	59
40	*rac*-LA/GL	12.5:12.5:1	CaLipase (50)	80	59	2100	1.19	0.12	72
41	*rac*-LA/GL	12.5:12.5:1	PcLipase (50)	80	45	1800	1.17	0.11	70
42	rac-LA/GL	12.5:12.5:1	PfLipase (50)	80	44	1700	1.22	0.11	81
43	*rac*-LA/GL	25:25:1	CaLipase (50)	60	22	1700	1.24	0.11	72
44	*rac*-LA/GL	25:25:1	CaLipase (50)	80	51	3200	1.32	0.14	75
45	*rac*-LA/GL	25:25:1	CaLipase (50)	100	53	3600	1.35	0.16	83
46	*rac*-LA/GL	50:50:1	CaLipase (50)	80	25	3400	1.37	0.15	79

Reaction conditions: time, 14 days
[a] Determined by GPC.
[b] Measured at 30°C in DMF.
[c] L_{CL} ($L_{rac\text{-LA}}$) (CL or *rac*-LA content in copolymer chain), determined by ^{1}H-NMR: L_{CL} (in PCLGL) = [Signal intensity of the —C(O)$CH_2CH_2CH_2CH_2CH_2$O—/Signal intensity of the —C(O)CH_2O—)·× 100; $L_{rac\text{-LA}}$ (in PLAGL) = [Signal intensity of the —OC(O)CH(CH_3)O—/Signal intensity of the —C(O)CH_2O—]·× 100.

to the Na^+ adduct (RM = 42 Da), whereas the second series of smaller peaks was also from cyclic PLA and corresponded to the Na^+ adduct (RM = 23 Da). In the MALDI–TOF MS spectrum of PLA, both populations of chains of even and odd numbers of lactic acid monomer unit (m.u.) could be observed. The odd number of acid m.u. showed that under the process conditions, the polymer chain underwent intermolecular transesterification (which led to an exchange of segments).

The mechanism was probably analogous to the mechanisms described in refs. [13,24], and [25] (enzyme-activated monomer). The initiation was a nucleophilic attack of PEG onto the acyl carbon of the intermediate to produce macrodiol. In the propagation stage, the intermediate was nucleophilically attacked by the terminal hydroxyl group of a propagating polymer to produce a one-unit-more elongated polymer chain. Kinetic and mechanistic studies of the e-ROP of cyclic monomers in the presence of PEG are underway. Detailed results will be presented in future articles.

Lipase-catalyzed ring-opening copolymerization of cyclic esters

In the next stage of the research work, the copolymerization reactions of CL, *rac*-LA, and GL catalyzed

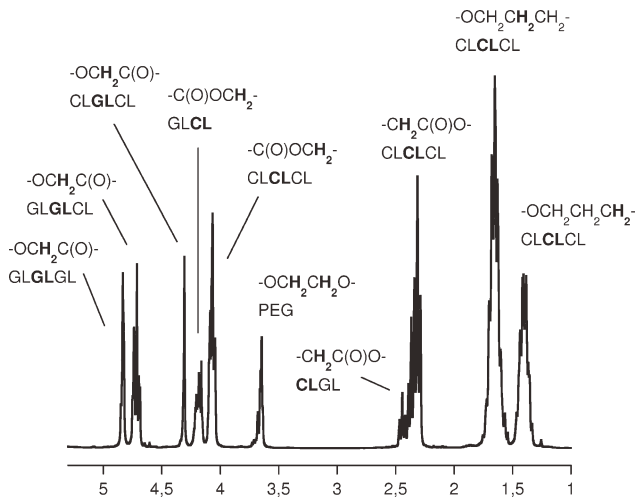


Figure 3 ^{1}H-NMR spectrum of PEG–PCLGL (in $CDCl_3$).

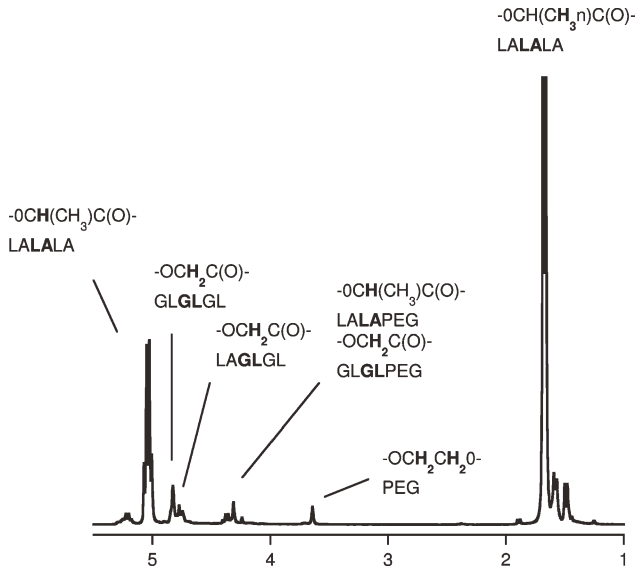


Figure 4 ^{1}H-NMR spectrum of PEG–PLAGL (in $CDCl_3$).

TABLE IV
^{1}H-NMR Structural Assignments of the Synthesized Copolymers (from Spectra Recorded in $CDCl_3$ at Room Temperature)

Chemical shift (ppm)	Structural assignment
1.37	—$OCH_2CH_2CH_2CH_2CH_2C(O)$—
1.64	—$OCH_2CH_2CH_2CH_2CH_2C(O)$—
2.36	—$OCH_2CH_2CH_2CH_2CH_2C(O)$—
2.43	—$OCH_2CH_2CH_2CH_2CH_2C(O)$—$OCH_2C(O)OCH_2C(O)$—
3.65	—OCH_2CH_2O—
4.05	—$OCH_2CH_2CH_2CH_2CH_2C(O)$—
4.18	—$OCH_2C(O)OCH_2C(O)$—$OCH_2CH_2CH_2CH_2CH_2C(O)$—
4.39	$OCH_2CH_2CH_2CH_2CH_2C(O)OCH_2C(O)$—$OCH_2CH_2CH_2CH_2CH_2C(O)$—
4.68	—$OCH_2C(O)OCH_2C(O)$—$OCH_2CH_2CH_2CH_2CH_2C(O)$—
4.82	—$OCH_2C(O)OCH_2C(O)$—
1.62	—$OCH(CH_3)C(O)$—
3.66	—OCH_2CH_2O—
4.40–4.20	—$OCH_2C(O)OCH_2C(O)$—OCH_2CH_2O—, —$OCH(CH_3)C(O)$—OCH_2CH_2O—
4.72	—$OCH(CH_3)C(O)$—$OCH_2C(O)$—$OCH_2C(O)$—
4.84	—$OCH_2C(O)OCH_2C(O)$—
5.12	—$OCH(CH_3)C(O)$—

by CaLipase, PcLipase, and PfLipase were examined. The polymerizations were carried out in bulk (CL/GL) or in toluene (*rac*-LA/GL) at 60–100°C for 14 days (Table III).

The chemical structures of the obtained copolymers were confirmed by ^{1}H-NMR (Figs. 3 and 4 and Table IV).

The influence of the comonomer/PEG feed ratio on the molecular weight of the copolyesters was studied at three levels (12.5:12.5:1, 25:25:1, and 50:50:1). As shown in Table III, the copolymers of CL and GL were obtained with M_n values (from GPC) of 1700–4400 Da. For the copolymers of *rac*-LA and GL, the M_n values (from GPC) amounted to 1700–3600 Da. Figure 5 shows typical GPC curves of the copolymer. All of the purified copolymers showed a single peak in the GPC curve. The molecular weight distribution of the polymer was rather narrow (1.17–1.37).

It was found that the molar mass of the copolymers increased with the comonomer/PEG feed ratio. On the other hand, according to the M_n values of the copolymers, the poly(ε-caprolactone–glycolide) (PCLGL) or poly(*rac*-lactide–glycolide) (PLAGL) yields had a tendency to decrease with increasing comonomer/PEG feed ratio. Both the yield and molecular mass of the copolymers increased when the reaction temperature was raised from 60 to 100°C. Similar behavior was observed for the lipase-catalyzed homopolymerization of CL and LLA or *rac*-LA.

The composition of the PCLGL and PLAGL copolymers was deduced from the ^{1}H-NMR spectra through the ratio of the peak areas corresponding to the LA [—$OC(O)CH(CH_3)O$—] protons at δ = 5.0–5.2 ppm, the CL [—$C(O)CH_2CH_2CH_2CH_2CH_2O$—] protons at δ = 4.0–4.2 ppm, and the GL [—$OC(O)CH_2O$—] protons at 4.80–4.85 ppm. The CL (or *rac*-LA) content in the copolymers exceeded the CL (or *rac*-LA) feed ratio for PCLGL (or PLAGL, which amounted to 57–69 and 70–83 mol %, respectively). Probably, CL and *rac*-LA were more active than GL in the copolymerization.

CONCLUSIONS

The enzyme-catalyzed ROP and copolymerization of CL, LLA, *rac*-LA, and GL, the effects of various catalyst concentrations on the reaction rates, and the molecular weights were studied. CaLipase gave higher yields than PcLipase or PfLipase in all of the reactions. The low-molecular weight PCL, PLA, and copolymers of CL and GL or *rac*-LA and GL, terminated at both sides by hydroxyl groups, were obtained by the ROP of cyclic esters in the presence of PEG/lipase systems. The possibility of using the

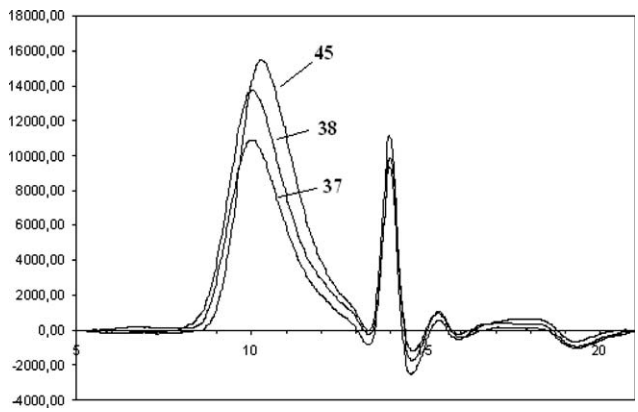


Figure 5 Eluograms of the obtained copolymers (the peaks at 14 and 12.5 mL represent ethanol and wax from the injection mold, respectively).

obtained biodegradable polyesters as carriers in drug-delivery systems is currently being studied.

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