Efficient Ring-Opening Polymerization and Copolymerization of ϵ-Caprolactone and ω-Pentadecalactone Catalyzed by *Candida antartica* Lipase B

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ABSTRACT: In this paper, Novozyme-435-catalyzed ω-pentadecalactone and ω-pentadecalactone/ϵ-caprolactone polymerizations were investigated. Novozyme-435-catalyzed ω-pentadecalactone polymerizations were studied in bulk and at ω-pentadecalactone-to-toluene ratios from 1:1 to 1:10 (wt/vol). By carrying out polymerizations with ω-pentadecalactone to toluene 1:1 wt/vol instead of in bulk, the monomer conversion (32 to 90%) and product M_n (22×10^3 to 86×10^3 g/mol) increased. Effects of reaction temperature on monomer conversion and product molecular weights also were studied. ω-Pentadecalactone polymerization at 90 °C in toluene (1:2 ω-pentadecalactone to toluene wt/vol) resulted in the fastest kinetics thus far reported for lipase-catalyzed polyester production. However, reduction of the polymerization temperature from 90 to 55 °C gave polypentadecalactone with increased M_n (66×10^3 to 81×10^3 g/mol). Novozyme-435-catalyzed ω-pentadecalactone/ϵ-caprolactone copolymerizations conducted at 70 °C in toluene occurred at unexpectedly rapid rates. Studies of monomer coreactivity ratios ($r_1 = 1.742$ and $r_2 = 0.135$) showed that ω-pentadecalactone reacted 13 times faster than ϵ-caprolactone. ^{13}C NMR studies showed that copolymers with random repeat unit sequence distributions were formed after 10 min at monomer conversions ≥44%). We believe that Novozyme-435 actively promotes interchain transesterification reactions that tend to randomize the repeat unit sequence distribution.

Introduction

The past 15 years have seen great progress in the use of enzymes in organic media to catalyze a wide variety of small molecule transformations.[1] The rationale for using enzymes as catalysts is described at great length elsewhere.[1−22] Our laboratory uses enzymes for polymer synthesis and modification reactions.[2−9] Lipases catalyze condensation[19−22] and lactone ring-opening polymerizations.[2,17] It is the latter area that is the topic of this paper.

Enzyme-catalyzed polymerizations reported between 1991 and 1999 using ϵ-caprolactone (CL),[16] δ-valerolactone (VL),[16] 8-octanolide,[10] β-propiolactone,[23] β-methyl-β-propiolactone,[24] (±)-α-methyl-β-propiolactone,[8] and γ-butyrolactone[23] as monomers required long reaction times (days) and produced low molecular weight polymers (M_n generally lower than 5×10^3 g/mol). Thus, even though lipase-catalyzed lactone polymerizations[5,8,24−26] are enzyme-selective (enantio- and regioselective phenomena), the long reaction times and low molecular weights may have diminished the importance of these findings. Research is needed to find new or improved methods for lipase-catalyzed polyester synthesis that provides favorable reaction kinetics and control of polymer structure.

Recently, our laboratory reported a model study using the monomer/catalyst system ϵ-caprolactone /Novozyme-435 (lipase B from *Candida antarctica*).[27] The propagation kinetics were improved by carrying out reactions in toluene (toluene to ϵ-caprolactone ratio of 2:1 wt/wt %). Some solvents also stabilize the lipase so that the preferred reaction temperature for rapid monomer polymerization is 90 °C. Thus, by adopting these reaction parameters, polycaprolactone monomer conversions of >80% and M_n values of >6000 g/mol were obtained within 1 h.

Traditional chemical catalysts polymerize four- to seven-membered lactones efficiently.[28] However, these catalysts do not have the inherent capabilities of enzymes to carry out selective transformations. In contrast to smaller cyclic lactones (four to seven-membered), macrolactones are difficult to polymerize by traditional chemical methods.[28] Polymerization of macrolactones proceed slowly and give low molecular weight polymers. Kobayashi and co-workers[13−15] were the first to investigate the enzyme-catalyzed polymerization of ω-undecanolide, ω-dodecanolide, and ω-pentadecanolide (12-, 13-, and 16-membered lactones). For example, they reported the synthesis of polydodecanolite with $M_n = 25 \times 10^3$ g/mol by reaction at 75 °C using the immobilized lipase from a *Pseudomonas* sp. (lipase PS, Toyobe Co.).[15] In our laboratory, the solventless polymerization of ω-pentadecalactone at 70 °C catalyzed by lipase PS-30 (immobilized on Celite) yielded polypentadecalactone in >90% with M_n 62×10^3 g/mol and M_w/M_n 1.9.[6] Our preliminary work also showed that Novozyme-435 from *Candida antarctica* may catalyze ω-pentadecalactone polymerization.[6]

Given our recent report of accelerated ϵ-caprolactone polymerizations in toluene, Novozyme-435-catalyzed ω-pentadecalactone polymerizations were revisited. Also, lipase-catalyzed copolymerizations have received little attention in the literature.[10,13,29] Lipase catalysis of copolymerizations may create highly ordered repeat-unit chain sequences. Alternatively, lipase catalysis may facilitate copolymerizations that are difficult using more traditional methods. Therefore, we studied the Novozyme-435/toluene system to copolymerize ω-pentadecalactone/ϵ-caprolactone, determined the monomer reactivity ratios at low conversion, and investigated the kinetic versus thermodynamic control of copolymer structure.

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Material and Methods

The monomer ω-pentadecalactone (PDL, 98%) (Aldrich Chemical Co.) was used as received and characterized by proton (^{1}H) nuclear magnetic resonance: ($CDCl_3$) δ 4.15 (2H, t), 2.35 (2H, t), 1.64 (4H, m) and 0.85 (22H, brs) ppm. ϵ-Caprolactone (polymerization grade) was obtained from Union Carbide Company. ϵ-Caprolactone was first dried over calcium hydride and distilled under reduced pressure in a nitrogen atmosphere. Anhydrous toluene and chloroform-*d* were purchased from Aldrich Chemical Co. Toluene was dried over calcium hydride and distilled under a nitrogen atmosphere. Coulomat A and Coulomat C were purchased from EM Science. Novozyme-435 (specified activity 7×10^3 PLU/g) was a gift from Novo Nordisk Co. All liquid chemical transfers were performed by syringe injection through rubber septa under a nitrogen atmosphere.

Novozyme-435-Catalyzed Ring-Opening Polymerization of ω-Pentadecalactone. Novozyme-435 (0.053 g) was dried in a vacuum desiccator (0.1 mmHg, 25 °C, 24 h) and transferred under nitrogen into oven dried 10 mL Pyrex culture tube containing ω-pentadecalactone (0.53 g). The vials were stoppered with rubber septa and sealed with Teflon tape. Toluene (1.06 mL) was subsequently added via syringe under nitrogen into the reaction vials. The vials were then placed into a constant temperature (for example, 70 °C) oil bath and shaken at 220 rpm for predetermined times (for example, 2 h). Reactions were terminated by adding excess of cold chloroform and removing the enzyme by filtration (glass-fritted filter, medium porosity). The filter residue was washed several times with hot chloroform, and the combined filtrates concentrated by rotary evaporation. The polymer in the concentrated filtrate was precipitated by the addition of methanol. The precipitate was isolated by filtration and dried in a vacuum oven (0.1 mmHg, 50 °C, 24 h). The polymer was isolated in 82% yields and had a M_n of 79×10^3 g/mol with PDI of 1.8, determined by GPC.

Novozyme-435-Catalyzed Copolymerization of ω-Pentadecalactone/ϵ-Caprolactone. The procedure for copolymerization of ω-pentadecalactone and ϵ-caprolactone was similar to the procedure mentioned above for ω-pentadecalactone polymerization, except comonomer ϵ-caprolactone was added along with ω-pentadecalactone in the reaction vials.

Instrumental Methods. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a DPX300 spectrometer at 300 and 75.13 MHz, respectively (Bruker Instrument, Inc.). The ^{1}H and ^{13}C NMR chemical shifts in parts per million (ppm) were referenced relative to tetramethylsilane (TMS) and chloroform as an internal standards. ^{13}C NMR spectra were recorded to determine the relative fractions of diad repeat unit sequences. The parameters used were as follows: 8.0% wt /wt polymer in $CDCl_3$, temperature 28 °C, pulse width 60°, 18 000 data points, relaxation delay 5.0 s, and 14 000−18 000 transients using a line broadening of 1 Hz.

Molecular weights were determined by gel permeation chromatography (GPC) using a Waters HPLC system equipped with model 510 pump, Waters model 717 autosampler, model 410 refractive index detector, and model T-50/T-60 detector of Viscotek Corp. with 500, 10^3, 10^4, and 10^5 Å Ultrastyragel columns in series. Trisec GPC software version 3 was used for calculations. Chloroform was used as the eluent at a flow rate of 1.0 mL/min at room temperature. Sample concentrations of 0.2% wt/vol and injection volumes of 100 μL were used. Molecular weights were determined on the basis of a calibration curve generated by narrow molecular weight polystyrene standards obtained from Aldrich Chemical Co.

Reaction initial water contents (wt % water) were measured by using an Aqua Star C 3000 titrator using Coulomat A and Coulomat C from EM Science. The water (wt/wt) in reaction mixtures were determined by stirring 53 mg of Novozyme-435, 1.68 g of toluene, and 0.53 g of monomer in Coulomat A, in a closed septum container designed for the instrument and titrating it against Coulomat C. The total water content (wt/wt) in the reactions was <0.8%.

Table 1. Effect of Monomer Concentration on Novozyme-435-Catalyzed Polymerization of PDL

product no.	toluene:monomer ratio (vol/wt)	% isolated yield	$M_n \times 10^{-3}$ (g/mol)[b]	PDI[a]
1	bulk	32	22	2.14
2	1:1	90	86	2.37
3	2:1	82	79	1.80
4	3:1	80	55	1.84
5	4:1	84	64	1.77
6	5:1	84	64	1.64
7	10:1	77	49	1.53

[a] Polymerization of PDL (0.5 g) catalyzed by Novozyme-435 (0.05 g) at 70 °C (PDL: toluene, 1:2, wt/vol) for 2 h. [b] Determined by GPC in $CHCl_3$.

Polypentadecalactone $-[-O{=}C-CH_2{}^b-CH_2{}^c-\{-CH_2{}^d-CH_2{}^d-\}_5-CH_2{}^c-CH_2{}^a-O-]-$ (M_n 81 × 10^3 g/mol and PDI 1.9). ^{1}H NMR ($CDCl_3$): δ 4.07 (t, J 6.5 Hz, $CH_2{}^aO$), 3.61 (t, J 6.5 Hz, CH_2OH), 2.31 (t, J 7.5 Hz, $CH_2{}^bCO$), 1.65 and 1.30 (brs, $CH_2{}^{c,d}$) ppm. ^{13}C NMR ($CDCl_3$): δ 173.9 (**C**OCH_2), 64.4 (**C**H_2aO), 34.4 (OCO**C**H_2b), 29.6−29.1, 28.6, 25.9, and 25.0 (all other carbons) ppm.

Poly(caprolactone-co-60 mol % pentadecalactone) $(-O{=}C-CH_2-CH_2\{-CH_2-CH_2-\}_5-CH_2-CH_2-O)-(O{=}C-CH_2-(CH_2)_2-CH_2-CH_2-O-)$. Isolated yield 34% in 5 min, $M_n = 14 \times 10^3$ g/mol, PDI = 2.37. ^{1}H NMR ($CDCl_3$): δ 4.07 (t, J 6.5 Hz, OCH_2), 3.61 (t, J 6.5 Hz, $HOCH_2$), 2.31 (t, J 7.5 Hz, $COCH_2$), 1.65 and 1.30 (brs, all other protons) ppm. ^{13}C NMR ($CDCl_3$): δ 173.87 (O**C**OCH_2, PDL*-PDL), 173.82 (O**C**OCH_2, PDL*-CL), 173.46 (O**C**OCH_2, CL*-PDL), 173.42 (O**C**OCH_2, CL*-CL), 64.44 (O**C**H_2, PDL*-CL), 64.35 (O**C**H_2, PDL*-PDL), 64.08 (O**C**H_2, CL*-CL), 63.96 (O**C**H_2, CL*-PDL), 34.40 (OCO**C**H_2, PDL*-PDL), 34.36 (OCO**C**H_2, PDL*-CL), 34.18 (OCO**C**H_2, CL*-PDL), 34.12 (OCO**C**H_2, CL*-CL), 29.61−29.12, 28.65 (**C**H_2 PDL), 28.3 (OCH_2**C**H_2, CL), 25.9 (**C**H_2 PDL), 25.5($OCOCH_2$**C**H_2, CL), 25.0 (**C**H_2 PDL) and 24.51($OCOCH_2CH_2$**C**H_2, CL) ppm.

Results and Discussion

Novozyme-435-Catalyzed Polymerization of ω-Pentadecalactone. Earlier reports[6,13] described effects of lipase and other reaction variables on lipase-catalyzed pentadecalactone polymerizations. For example, the molecular weight of polypolypentadecalactone obtained by bulk (solventless) polymerization at 75 °C gradually increased as a function of time (72−720 h, $M_n = (9-20) \times 10^3$ g/mol)[13] using *Candida cylindracea* lipase (20% lipase with respect to ω-pentadecalactone). Earlier, we demonstrated that by using Celite-immobilized lipase PS-30 (25% lipase with respect to ω-pentadecalactone) in bulk the molecular weight of polypentadecalactone decreased with increase in reaction time from 1 to 48 h (35−100% monomer conversion, M_n 62 000−50 000 g/mol). Stimulated by our recent report that Novozyme-435 could be used to accelerate ϵ-caprolactone polymerizations in low-polarity organic media,[27] work was initiated to determine the extent that ω-pentadecalactone polymerization also could be regulated in this lipase/solvent polymerization system.

Ratios of toluene to PDL of 0:1 (solventless), 1:1,2:1,3:1,4:1,5:1,10:1 (vol/wt), were investigated for polymerizations at 70 °C for 2 h (see Table 1). The addition of toluene increased the percent monomer conversion, product M_n, and polydispersity index (PDI). For example, the polypentadecalactone yield and M_n increased from 32 to 90% and 22 000 to 86 400 g/mol, respectively, when the polymerization was conducted at low toluene levels (toluene/ω-pentadecalactone 1:1 vol/wt) compared to bulk reactions. In general, the M_n and PDI decreased when the ratio of toluene to ω-pentadecalactone was

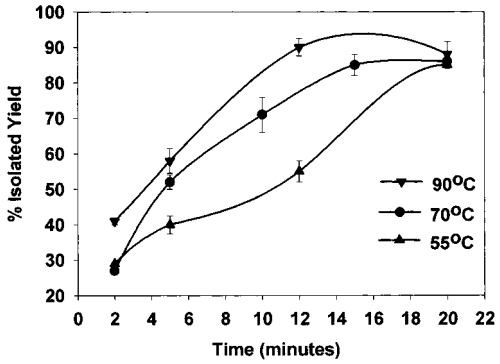

Figure 1. Plot of isolated yield vs reaction time for Novozyme-435 (PDL to Novozyme-435, 1:10 wt/wt) catalyzed polymerization of ω-pentadecalactone at 90, 70, and 55 °C in toluene (toluene to ω-pentadecalactoneat, 2:1, vol/wt).

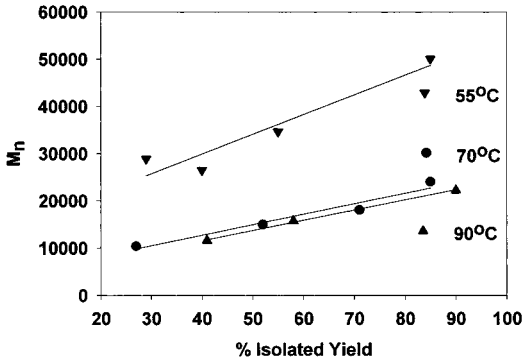

Figure 2. Plot of M_n vs isolated polypentadecalactone yield for Novozyme-435 (ω-pentadecalactone at to Novozyme-435, 1:10 wt/wt) catalyzed polymerization of ω-pentadecalactone at 90, 70, and 55 °C in toluene (toluene to ω-pentadecalactone, 2:1, vol/wt).

greater then 1:1 (Table 1). For example, the PDI decreased from 2.37 to 1.53 as the ratio of toluene to ω-pentadecalactone increased from 1:1 to 10:1 (vol/wt). An increase in the diffusivity of the propagating chain and monomer is in part responsible for the observed increase in yield and molecular weight for solution polymerizations of pentadecalactone catalyzed by Novozyme-435. The toluene-to-pentadecalactone ratio of 2:1 was selected for further studies since it resulted in polypentadecalactone of similar M_n but decreased PDI than when toluene-to-pentadecalactone 1:1 was used. Decrease in the PDI with increase in toluene to ω-pentadecalactone ratio may be due to the decrease in transesterification of polypentadecalactone chains. Increasing the scale of the reaction from 0.5 to 60 g, ω-pentadecalactone was nonproblematic, giving the product in similar molecular weight and higher isolated yield (93% as compared to 78%).

The effect of carrying out ω-pentadecalactone polymerizations at various temperatures also was studied. Maintaining the toluene/ω-pentadecalactone ratio at 2:1 (vol/wt), ω-pentadecalactone polymerizations were conducted at 55, 70, and 90 °C for 2 to 20 min (Figure 1) and 4 h. The rate of polymerization increased from 55 to 90 °C. For example, at 90 °C, within only 2 and 5 min, the product yield reached 41 and 58%, respectively. In contrast, at 70 and 55 °C, the product yields after 2 and 5 min were 27 and 28%, respectively, and 52 and 40%, respectively (Figure 1). Uyama and co-workers[13,14] demonstrated that the rate of ω-pentadecalactone polymerization catalyzed by lipases PF (*Pseudomonas fluorescens*) and CC (*Candida cylindracea*) increased with increasing reaction temperature (45−60 °C), although increasing the reaction temperature from 60 to 75 °C did not increase the product yields. Other research groups have not explored lipase-catalyzed ω-pentadecalactone polymerizations at reaction temperatures above 75 °C. Previously, our laboratory reported that ω-pentadecalactone polymerizations in bulk catalyzed by lipase PS immobilized on Celite increased ω-pentadecalactone conversion from 80 to 91% in 1 h with increase in temperature from 60 to 70 °C.[6] However, under identical reaction conditions, similar monomer conversion resulted when the reaction temperature was increased from 70 to 90 °C.[6] Thus, the temperature−yield relationship for lipase-catalyzed ω-pentadecalactone polymerizations varies as a function of the enzyme and reaction conditions used. Such variations are explained by inherent differences in the thermal stability of different lipases. Furthermore, lipase B from *Candida antartica* has been stabilized by immobilization and by the introduction of toluene into the polymerizations. Novozyme-435-catalyzed polymerizations of ϵ-caprolactone under identical reaction conditions as described herein also showed increased product yields as the reaction temperature was increased from 60 to 70 to 90 °C.[27] However, the relative rates of ω-pentadecalactone and ϵ-caprolactone polymerization are very different. For example, at 90 °C after 5 and 15 min, the isolated product yield of polycaprolactone reached 4% and 12%, respectively. In contrast, the isolated product yields of polypentadecalactone under identical conditions were 58% and 90%, respectively.

Figure 2 shows that at 55, 70, and 90 °C the polypentadecalactone M_n increased linearly as a function of monomer conversion. This linear relationship between M_n and conversion was seen previously for ϵ-caprolactone polymerization in toluene-d_8 at temperatures between 60 and 85 °C,[27] suggesting that chain-transfer reactions are absent. The M_n values were highest at 55 °C and decreased as the temperature was increased to 90 °C. Previous work describes very different relationships between reaction temperature and product M_n.[13,14] For example, Kobayashi and co-workers[14] reported that with decrease in reaction temperature from 75 and 60 °C (ω-pentadecalactone ring-opening polymerizations catalyzed by lipase CC and PF) polypentadecalactone M_n decreased from 9500 to 4600 and 7200 to 2700 g/mol, in 72 and 120 h, respectively. Similarly,[13] for undecanolactone (UDL) polymerization catalyzed by lipase PF, the polymer M_n decreased when the reaction temperature was decreased from 75 to 60 to 45 °C (19 500 to 8500 to 2800 g/mol in 48 h). Thus, the temperature−M_n relationships for lipase-catalyzed ω-pentadecalactone can even vary inversely when comparing similar reactions conducted using different lipases. Previously we described how M_n increases as the water content decreases.[6] Reports by us[7,9] and others[14,15] agree that water functions as the initiator by reacting with an enzyme activated monomer complex to form the corresponding ω-hydroxyalkanoic acid. Accordingly, in the water content of enzyme preparations or in the reaction medium should increase the number of chains and decrease M_n.[6,30,31] Increase in the water concentration would lead to the initiation of a greater number of chains and hence polymer with lower M_n. In addition, in bulk polymerization restricted diffusion of propagating chains and monomer can limit the extent that higher molecular weight chains can further grow. Accordingly,

Table 2. Novozyme-435-Catalyzed Copolymerization of PDL and CL

no.	$[PDL]_0/[CL]_0$	time	% yield	CL/PDL obs, mol %	CL*-CL obs (cal)[a]	CL*-PDL obs (cal)[a]	PDL*-CL obs (cal)[a]	PDL*-PDL[a] obs (cal)	M_n[c] $\times 10^2$, g/mol	M_w/M_n[c]
1	4:1	1 min	22	15/85	0.11 (0.02)	0.03 (0.13)	0.11 (0.13)	0.75 (0.72)	92	2.80
2	2:1	1 min	20	21/79	0.15 (0.04)	0.05 (0.17)	0.15 (0.17)	0.64 (0.62)	94	2.59
3	1:2	1 min	09	35/65	0.16 (0.12)	0.18 (0.23)	0.18 (0.23)	0.45 (0.42)	85	2.26
4	1:4	1 min	09	55/45	0.55 (0.30)	0.24 (0.25)	0.20 (0.25)	0.25 (0.21)	112	2.11
5	1:8	1 min	04	60/40	0.40 (0.36)	0.20 (0.24)	0.17 (0.24)	0.23 (0.16)	63	2.47
6	1:15	1 min	04	67/33	0.49 (0.45)	0.18 (0.22)	0.16 (0.22)	0.17 (0.11)	73	2.14
7	1:1	1 min	19	31/69	0.11 (0.10)	0.20 (0.21)	0.19 (0.21)	0.50 (0.47)	84	2.50
8	1:1	2 min	23	37/63	0.14 (0.14)	0.23 (0.23)	0.20 (0.23)	0.42 (0.40)	126	2.19
9	1:1	5 min	34	40/60	0.19 (0.16)	0.21 (0.24)	0.23 (0.24)	0.37 (0.36)	146	2.37
10	1:1	10 min	44	48/52	0.25 (0.21)	0.24 (0.25)	0.21 (0.25)	0.31 (0.29)	168	2.41
11	1:1	15 min	53	50/50	0.26 (0.25)	0.24 (0.25)	0.22 (0.25)	0.28 (0.25)	178	2.38
12	1:1	30 min	72	49/51	0.26 (0.24)	0.23 (0.25)	0.25 (0.25)	0.26 (0.26)	198	2.46
13	1:1	45 min	88	50/50	0.28 (0.25)	0.22 (0.25)	0.26 (0.25)	0.24 (0.25)	193	2.28
14	1;1	1 h	88	49/51	0.29 (0.25)	0.20 (0.25)	0.23 (0.25)	0.27 (0.25)	196	2.37
15	1:1	2 h	89	50/50	0.25 (0.25)	0.25 (0.25)	0.23 (0.25)	0.27 (0.25)	180	2.08
16	1:1	4 h	90	52/48	0.23 (0.26)	0.30 (0.25)	0.24 (0.25)	0.24 (0.24)	212	1.87
17	1:1	6 h	93	52/48	0.26 (0.26)	0.26 (0.25)	0.24 (0.25)	0.24 (0.24)	223	1.97
18	1:1	24 h[d]	87	53/47	0.31 (0.28)	0.23 (0.25)	0.21 (0.25)	0.26 (0.22)	183	1.97

[a] Copolymerization of ω-pentadecalactone and ϵ-caprolactone catalyzed by Novozyme-435 (1/10 wt/wt, with respect to monomer) in toluene (2 times vol/wt of monomers) at 70 °C. [b] PDL unit next to PDL is given as PDL*-PDL = P^2_{PDL}, PDL next to CL or CL next to PDL is given as PDL*-CL = CL*-PDL = $2P_{PDL}(1 - P_{PDL})$, similarly CL next to CL is given as CL*-CL = $(1 - P_{PDL})^2$. [c] Determined by GPC in $CHCl_3$. [d] CL added after 3 h of polymerization of PDL.

in this work, where polymerizations were conducted in toluene, more likely diffusion may not be limiting so that increases in reaction temperature liberate water and reduce M_n. However, in the other cases described above[6,13,14] the polymerizations were carried out in the absence of solvent (bulk). It is likely that for bulk polymerizations diffusion limits chain growth so that the dominant effect of increased temperature is increased diffusion that results in increased M_n.

Other considerations that may influence the temperature−M_n relationships are as follows. For some enzyme−monomer systems, an increase in water content may lead enzyme-catalyzed chain hydrolysis.[32] Thus, relationships between M_n and reaction temperature may be determined by many factors that will differ in importance as a function of the enzyme−monomer−solvent polymerization system.

Calculation of Turnover Rates. To better appreciate how rapid ω-pentadecalactone ring-opening polymerizations take place in organic media, a comparison was made on the turnover values for this versus a routine lipase-catalyzed transformation with low molecular weight substrates. This calculation was made by taking the value of 10% for the protein content in Novozyme-435, assuming that 100% of the protein in Novozyme-435 is lipase B and assuming that there is no denaturation during enzyme immobilization.[33] In addition, the molecular weight of lipase B is known (33 kDa).[34] For this calculation, we selected the result that 31% conversion of ω-pentadecalactone to polypentadecalactone occurred within 1 min (90 °C, in toluene). Our estimate of the turnover rate of ω-pentadecalactone to polypentadecalactone under these reaction conditions is 4.23 mmol PDL min^{-1} μmol lipase B^{-1}. For low molecular weight substrates, Novozyme-435-catalyzed esterification of palmitic acid with octadecenol was used to calculate turnover values. The best result obtained was 13% esterification of palmitic acid in 2 min (70 °C, in toluene). Our estimate of the turnover rate of palmitic acid to octadecenylpalmitate under these reaction conditions is 0.054 mmol palmiticacid min^{-1} μmol lipase B^{-1}. Novozyme-435-catalyzed ω-pentadecalactone polymerization in toluene was found to be 78 times faster than the above system.

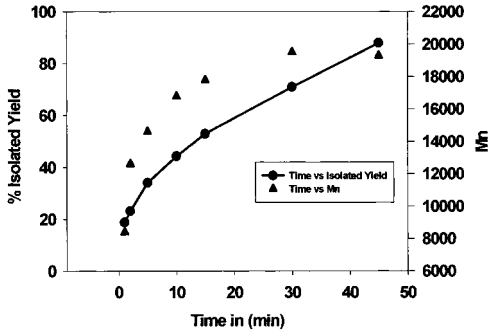

Figure 3. Plot of M_n vs reaction time and isolated yield of copolymers formed by Novozyme-435-catalyzed copolymerization of ϵ-caprolactone and ω-pentadecalactone at 70 °C in toluene (toluene to ω-pentadecalactone, 2:1, vol/wt).

To confirm that the turnover rate reported above for ω-pentadecalactone polymerization at 90 °C was due to enzyme catalysis, control reactions were performed. These reactions were carried out at 90 °C in toluene solution (toluene/PDL 2:1) by (a) omission of the catalyst from the reaction mixture, (b) addition of the immobilization matrix that did not contain adsorbed enzyme, and (c) addition of deactivated Novozyme-435 to the reaction mixture. In all of these circumstances, no polymer precipitate was observed by precipitation with methanol. Furthermore, no evidence for the conversion of ω-pentadecalactone to oligomers was observed based on ^{1}H NMR analyses of the reaction mixtures.

Novozyme-435-Catalyzed Copolymerization of ω-Pentadecalactone with ϵ-Caprolactone. Novozyme-435-catalyzed ω-pentadecalactone/ϵ-caprolactone copolymerizations were conducted in toluene at 70 °C in solutions containing a toluene-to-monomer ratio of 2:1 (vol/wt). The monomer feed ratios and reaction times for the copolymerizations are listed in Table 2. Copolymerization of ω-pentadecalactone and ϵ-caprolactone with a 1:1 molar feed ratio were carried out for reaction times from 1 min to 6 h (Table 2). Increase in the reaction time from 1 to 45 min resulted in a increase in product yield and M_n (19−88% and 8410−19 000 g/mol, respectively, Figure 3). Further increase in reaction time from 45 min to 6 h resulted in small increases in the yield and M_n from 88 to 93% and from 19 300 to 22 300

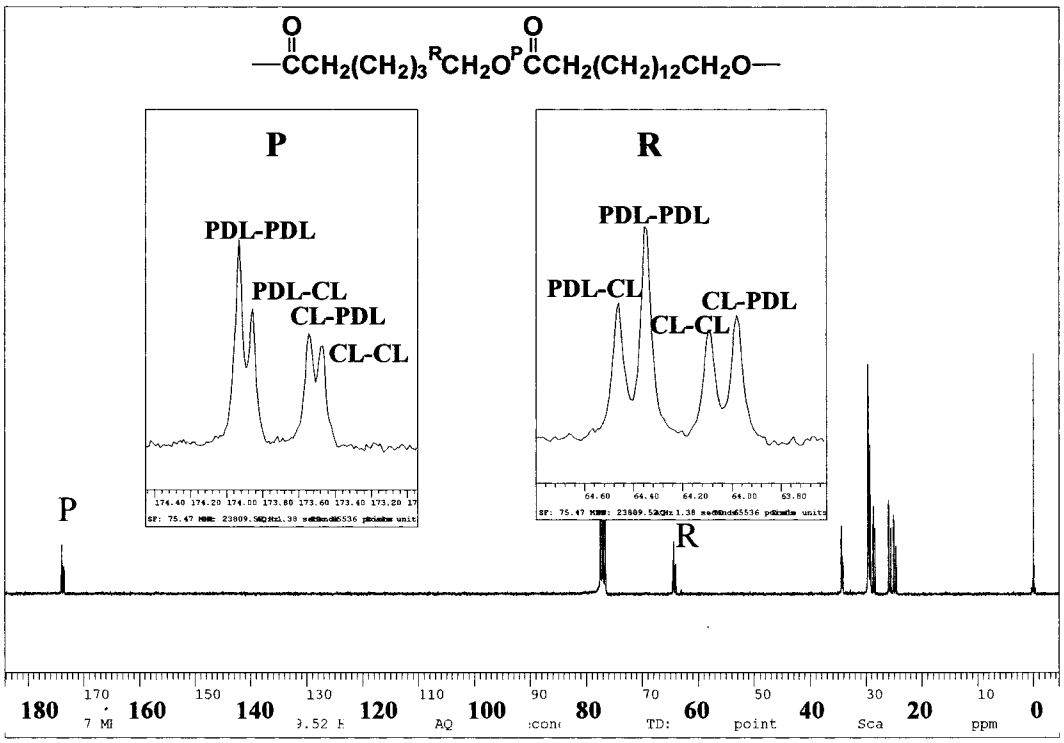

Figure 4. ^{13}C NMR spectrum at 27 °C in $CDCl_3$ of the poly(CL-*co*-60 mol % PDL) (product 9, Table 2) formed by Novozyme-435-catalyzed copolymerization of ϵ-caprolactone and ω-pentadecalactone (1:1) after 5 min.

Scheme 1. Possible Diads and Carbons Observed for Copolymers from PDL and CL

$COCH_2(CH_2)_{12}{}^{R}CH_2O{}^{P}COCH_2(CH_2)_{12}CH_2O$	PDL*-PDL
$COCH_2(CH_2)_3{}^{R}CH_2O{}^{P}COCH_2(CH_2)_{12}CH_2O$	CL*-PDL
$COCH_2(CH_2)_{12}{}^{R}CH_2O{}^{P}COCH_2(CH_2)_3CH_2O$	PDL*-CL
$COCH_2(CH_2)_3{}^{R}CH_2O{}^{P}COCH_2(CH_2)_3CH_2O$	CL*-CL

g/mol, respectively. Also, increase in reaction time from 1 to 45 min resulted in an increase in polymer caprolactone content (31−50 mol %). This result is a consequence of initial faster rate while ω-pentadecalactone is converted into copolymer. The structure of the ω-pentadecalactone/ϵ-caprolactone copolymers was analyzed by ^{13}C NMR spectroscopy. Figure 4 shows the complete carbon spectrum of poly(CL-*co*-60 mol % PDL) (product 9, Table 2) with expanded spectral regions from δ 173.0−175.0 and 63.6−65.0 of the multiple peaks corresponding to $O^{P}COCH_2$ and $^{R}CH_2O$, respectively. The four possible diad arrangements (CL*-CL, CL*-PDL, PDL*-PDL, and PDL*-CL) and assignment of the observed $^{R}CH_2O$ and $O^{P}COCH_2$ signals within these diads are shown in Scheme 1. The assignment of the diads was based on a series of carbon experiments performed by adding 50 mol % of one of the homopolymers (polycaprolactone or polypentadecalactone PDL) to poly(CL-*co*-50 mol % PDL) (product 15, Table 2). For example, when 50 mol % polycaprolactone was added to poly(CL-*co*-50 mol % PDL), increase in the intensity of signals at δ 173.42, 64.08, and 34.15 relative to the signals in the spectrum of poly(CL-*co*-50 mol % PDL) allowed the assignment of $O^{P}COCH_2$ and $^{R}CH_2O$ signals due to CL*-CL diads. Using the identical strategy, 50 mol % polyPDL was added to poly(CL-*co*-50 mol % PDL), and then the ^{13}C NMR spectrum was recorded. This resulted in increased intensity of the signals at δ 174.87, 64.35, and 34.36 that facilitated assignments of PDL*-PDL diads. The ^{13}C NMR signals corresponding to mixed diads (CL*-PDL and PDL*-CL) were assigned on the basis of the assumption that signals due to the CL*-PDL diads will be in close proximity to CL*-CL diads. Similarly, it was assumed that ^{13}C NMR signals due to PDL*-CL diads would be found at a spectral position that was in close proximity to PDL*-PDL diads. The assignments of the four-diad possibilities are shown in Figure 4. Comparison of the spectral regions δ 173.0−175.0 and δ 63.6−65.0 shows that the latter gives signals that are better resolved. Thus, observation of the expanded region from δ 63.6 to 65.0 due to $^{R}CH_2O$ was used to determine the observed diad fraction values (Table 2). Calculations of diad fraction values were based on a series of equations (see ref 35) that assume a Bernoulli or random statistical copolymerization of the two monomers. The polymer formed by copolymerization of ω-pentadecalactone/ϵ-caprolactone (1:1 mol/mol) in <5 min reaction time showed slight deviation in observed and calculated diad fractions, whereas products after 10 min (see Table 2) showed excellent agreement between observed and calculated diad fractions.

The formation of random copolymers can be explained by that, when a ϵ-caprolactone or ω-pentadecalactone repeat unit is at the growing chain terminus, either of the incoming monomers can add with a probability approaching equality to form a random copolymer. However, based on the increase from 31 to 50 mol % in the copolymer caprolactone content for reaction times from 1 to 45 min (1:1 monomer feed ratio), ω-pentadecalactone must be converted more rapidly than caprolactone into the copolymer. To determine the reactivity ratios, a series of copolymerizations with different monomer feed ratios were carried out for 1 min to ensure that monomer conversions remained <10%. The compositions of the polymers were determined by ^{13}C NMR analyses (see Table 2). The reactivity ratios were then determined based on the Fineman−Ross method by

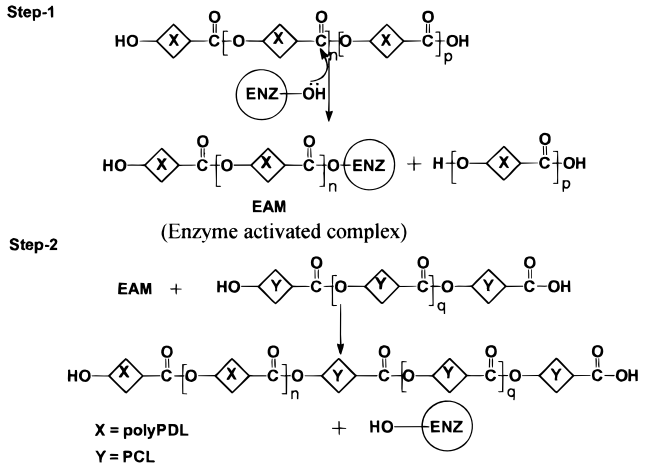

Figure 5. Mechanism of lipase-catalyzed transesterification reaction.

plotting F^2/f (x-axis) vs $F/f \times (f - 1)$ (y-axis) where F is the monomer feed ratio and f is the copolymer repeat unit composition (mol/mol) (see the Supporting Information to view this plot).[36] The reactivity rate of ω-pentadecalactone polymerization ($r_1 = 1.742$) was 13 times larger than for ϵ-caprolactone polymerization ($r_2 = 0.135$). Despite the large difference in the reactivity ratios for ω-pentadecalactone and ϵ-caprolactone copolymerization, the products isolated had repeat unit sequence distributions that approximated that of random copolymers. Furthermore, GPC traces of the copolymer series from the 1:1 monomer feed ratio all had distributions that were unimodal. In another experiment, ω-pentadecalactone was polymerized as above for 3 h, which should be sufficient to form the homopolymer in high yield (see Table 2, no. 15). Subsequently, ϵ-caprolactone was added to the reaction vessel to simulate an initial monomer feed ratio of 1:1. The reaction was maintained at 70 °C with agitation for 21 h, and then the polymer was isolated. The product poly(CL-*co*-47 mol % PDL) (Table 2, no. 18) had an M_n of 18 300 g/mol and polydispersity index of 1.9. Comparison of the observed and calculated diad fraction values shows that they are in excellent agreement. Thus, the addition of ϵ-caprolactone to a preformed polypentadecalactone gave a product that was random. This finding, in combination with the formation of random copolymers even though ω-pentadecalactone reacts much more rapidly than ϵ-caprolactone, shows convincingly that in addition to catalyzing chain propagation, Novozyme-435 also promotes rapid transesterification reactions between chains. A proposed mechanism for lipase-catalyzed transesterification reactions is shown in Figure 5.

Summary of Results

Increase in the solvent-to-monomer ratio from 1:1 to 10:1 (toluene:ω-pentadecalactone) decreased both polypentadecalactone M_n and polydispersity index (86 400 to 48 600 g/mol and 2.37 to 1.53, respectively). For most of the experiments in this paper, the toluene to ω-pentadecalactone ratio was 2:1. The polymerization of ω-pentadecalactone at 90 °C (toluene to ω-pentadecalactone 2:1) resulted in the fastest kinetics (4.23 mmol PDL min^{-1} μmol lipase B^{-1}) thus far reported for a lipase-catalyzed polyester synthesis. By reducing the polymerization temperature from 90 to 70 to 55 °C the polymerization rate decreased but the polypentadecalactone M_n increased from 65 700 to 79 400 to 81 300 g/mol, respectively. Novozyme-435-catalyzed copolymerization of ϵ-caprolactone and ω-pentadecalactone at 70 °C (toluene to PDL 2:1 vol/wt) occurred rapidly. In only 45 min, a copolymer in 88% yield was formed that had an M_n of 20 000 g/mol. Comparison of the copolymer composition from 1 and 45 min reaction times (monomer feed ratio 1:1) showed that the content of ϵ-caprolactone in the copolymer increased from 31 to 50 mol %. Thus, ω-pentadecalactone was initially more rapidly converted into the copolymer. Experimental determination of the reactivity ratios showed that ω-pentadecalactone polymerization ($r_1 = 1.742$) was 13 times faster than ϵ-caprolactone polymerization ($r_2 = 0.135$). Despite the large difference in the reactivity of these monomers, random copolymers were formed as a result of Novozyme-435 transesterification between the chains. Considering the requirement that two macromolecules must be in close proximity at the lipase active site to carry out the transesterification reaction, it is surprising that such reactions appear to occur between the ω-pentadecalactone/ϵ-caprolactone copolymer chains. Furthermore, the potential of applying lipase catalysis for a range of low-temperature transesterification reactions opens up a number of new opportunities in macromolecular synthesis. Further work is underway to extend this finding to other systems, to better understand the factors that promote and disfavor the transesterification pathway, and to study the mechanism of these reactions.

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Supporting Information Available: Fineman−Ross plot. This material is available free of charge via the Internet at http://pubs.acs.org.

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