Enzyme-Catalyzed Ring-Opening Polymerization of ω-Pentadecalactone†

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ABSTRACT: Lipase-catalyzed ring-opening bulk polymerizations of ω-pentadecalactone (PDL) were investigated. Screening of selected commercial lipases as catalysts for PDL polymerization at 80 °C was carried out. The results of this work showed that polymerizations catalyzed by lipases PS-30, AK, Lipozyme-IM and Novozym-435 gave % PDL conversions ranging from 80 to 100% for 24 h reactions (M_n = 15 000−34 400). Lipase PS-30 both physically immobilized onto Celite-521 (I-PS-30) and in the crude powder or nonimmobilized form (NI-PS-30) was selected for further study. Comparison of % conversion vs time for bulk PDL polymerizations at 70 °C catalyzed by NI- and I-PS-30 showed that for short reaction times, the immobilized catalyst gave % conversions that were more than 10 times greater. In fact, the % monomer conversion to poly(PDL) was nearly quantitative (>98%) for 8 h polymerizations catalyzed by I-PS-30. Furthermore, for reactions conducted at 70 °C with careful removal of water, substantially greater poly(PDL) molecular weights resulted by using I-PS-30 instead of NI-PS-30 as the catalyst. Increasing the % conversion above ∼40% for PDL polymerizations at 70 °C resulted in little or no change in PDL M_n. This is consistent with chain polymerizations where the rate of propagation is much faster than initiation. The general trends observed by variation of the I-PS-30 catalyzed bulk PDL polymerization temperature were the following: (*i*) increased % conversion and M_n by increasing the reaction temperature from 60 to 70 °C and from 60 to 80 °C, respectively, (*ii*) similar polymerization rates between 70 and 90 °C, and (*iii*) a decrease in % monomer conversion and M_n as the reaction temperature was increased from 90 to 110 °C. It was found that water was an important factor that controls not only the rate of monomer conversion but also the polymer molecular weight. From an increase in the water content in reactions, enhanced polymerization rates were achieved while the molecular weight of poly(PDL) decreased. At low reaction water levels (0.20% w/w water), the I-PS-30 catalyzed polymerization of PDL at 70 °C gave poly(PDL) with M_n and M_w/M_n of 62 000 and 1.9, respectively. Thus far, this is the highest molecular weight polyester prepared by an enzyme-catalyzed polymerization reaction.

Introduction

Suspensions of enzymes in organic media were shown to be powerful catalysts for a wide range of small molecule stereo- and regioselective organic transformations.[1−3] Furthermore, enzymes represent a family of "environmentally friendly" catalysts. Lately, the use of enzymes for polymer-forming reactions has gained increasing attention. Polymer synthesis by various strategies including chemo-enzymatic methods have been reported.[4−7] Early work by Margolin *et al.*[8] and Wallace *et al.*[9] showed that enzyme-catalyzed transesterification reactions between diesters and diols could be used to prepare chiral low molecular weight polyesters. Other work directed toward enzyme-catalyzed condensation reactions was summarized elsewhere.[10] While much progress has been made, these polymerizations generally involved long reaction times and gave low molecular weight products.

Ring-opening chemistry offers an interesting route to enzyme-catalyzed polymerizations as no leaving group is generated during the course of these reactions which can limit monomer conversion or degree of polymerization.[11] The 4-, 6-, and 7-membered lactones can be polymerized by various chemical initiators or catalyst systems to prepare high molecular weight polyesters with rapid propagation kinetic.[12] Considering the above stated advantages of enzyme-catalyzed organic reactions, research has been conducted on enzyme-catalyzed ring-opening polymerizations of ϵ-caprolactone (ϵ-CL),[7,11,13] δ-valerolactone(δ-VL),[7] β-propiolactone,[14] β-methyl-β-propiolactone,[14] (±)-α-methyl-β-propiolactone[10] and γ-butyrolactone.[14] The enzyme-catalyzed copolymerization of β-propiolactone with ϵ-CL was also investigated.[15] These polymerizations were reported to give low molecular weight products after long reaction times. The enzyme-catalyzed polymerization of (±)-α-methyl-β-propiolactone proceeded by the enantiomorphic site model to give an enantioenriched polymer.[10] It is also noteworthy to mention that enzyme-catalyzed ring-opening can be used to prepare enantioenriched monomers which, subsequently, can be polymerized by chemical methods. Examples that demonstrate this chemo-enzymatic strategy include work carried out by Xu *et al.* for α-methyl-β-propiolactone[16] and β-methyl-β-propiolactone.[17]

To improve the molecular weight and propagation kinetics of enzyme-catalyzed ring-opening polymerizations, we investigated the propagation kinetics and mechanism of porcine pancreatic lipase (PPL) catalyzed

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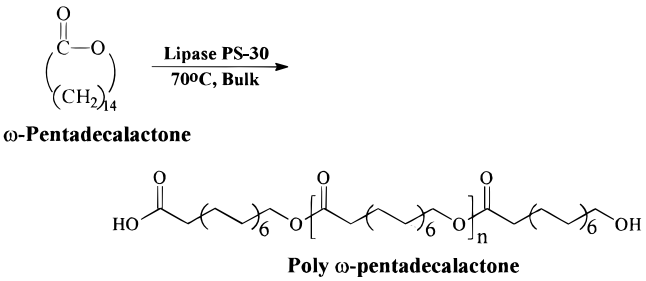
ϵ-CL polymerizations.[18] Assessment of $\log\{[M]_o/[M]_t\}$ vs time and M_n vs conversion indicated that termination and chain transfer did not occur. Therefore, the system provided "controlled" polymerizations where the molecular weight was a function of the monomer to initiator stoichiometry.[18]

In contrast to 4-, 6-, and 7-membered lactone polymerizations, the polymerization of macrolactones by traditional chemical methods proceeded slowly and gave low molecular weight polymers.[19] Interestingly, enzyme-catalyzed polymerizations of macrolactones have in some cases proved advantageous relative to chemical preparative routes. The enzyme-catalyzed polymerization of ω-undecanolide (UDL), ω-dodecanolide (DDL), and ω-pentadecanolide (PDL) (12-, 13-, and 16-membered lactones) were first investigated by Kobayashi and co-workers.[20−22] Screening of enzymes for the polymerization of UDL, DDL and PDL using lipases including those from *Aspergillus niger, Candida cylindracea* (lipase B), *Candida rugosa, Rhizopus delmar, Rhizopus javanicus, Pseudomonas fluorescens* (lipase P, Cosmo Bio.), and *Pseudomonas* sp. (lipase PS, Amano) as well as phospholipase and porcine pancreatic lipase was carried out.[20−22] Quantitative conversions of UDL to poly(UDL) were achieved within 120 h using lipase P and PS.[20] The highest number average molecular weight (M_n = 25 000) reported by these workers was for poly(DDL) synthesis (75 °C, 120 h) using the immobilized lipase PS from a *Pseudomonas* sp. (lipase PS, Toyobe Co.).[21] However, the effects of enzyme immobilization was not determined since the same crude enzyme powder was not investigated.

Enzyme immobilization can result in improved enzyme stability, recyclability, and activity for esterification reactions.[23] The effects of enzyme loading and additives on the activity of α-chymotrypsin and lipase P (from *Pseudomonas flourescens*) deposited on solid supports were studied by Wehtje and co-workers.[24] Interestingly, the adsorption of lipase PS-30 on Celite was used for resolution of alcohols by esterification/transesterification reactions.[25] A description of alternative techniques for immobilization of enzymes were described elsewhere.[24−29]

Our laboratories have an ongoing program to exploit the unique properties of enzymes as catalysts for polymer synthesis, modification and structural "tailoring".[5,10,13,18,30] The polymerization of PDL was of particular interest to us since it gives a polyester that would likely have properties that approach polyethylene. Furthermore, this polymer has ester functionalities along the chain which may allow poly(PDL) to degrade by microbial metabolism in various environments. In this paper, we first screened lipases not previously used for PDL polymerization and found that PS-30 from *Pseudomonas* sp. was best suited. We then used lipase PS-30 (nonimmobilized and immobilized on Celite-521) for investigations of PDL ring-opening polymerization in bulk (Scheme 1). Effects of PS-30 immobilization on PDL conversion and product molecular weight was evaluated as a function of (*i*) reaction time, (*ii*) reaction temperature (60−110 °C), and (*iii*) enzyme water content. Immobilization of PS-30 was used to dramatically increase propagation rates. In addition, water was used as a critical reaction parameter which altered propagation rates as well as product molecular weight. This study established polymerization conditions by which rapid PDL conversion to polymer and high molecular weight poly(PDL) was achieved.

Scheme 1



Experimental Section

Materials. The monomer ω-pentadecalactone (PDL, 98%) was purchased from Aldrich, used without further purification, and characterized by proton (^{1}H) nuclear magnetic resonance: 4.15 (2H, t), 2.35 (2H, t), 1.64 (4H, m), and 0.85 (22H, br s) ppm. Celite-521, also purchased from Aldrich, was washed thoroughly with distilled water and 0.1 M phosphate buffer (pH 7.0). All other chemicals used were of analytical grade and were used as received. The lipases PS-30 (*Pseudomonas* sp.), AK (*Pseudomonas* sp.) and MAP-10 (*Mucor* sp.) were obtained from Amano enzymes (USA) Co., Ltd (specified activity 30 000, 20 000, and 10 000 u/g, respectively at pH 7.0; a unit is the amount of enzyme which releases 1 μmol of fatty acid/min from olive oil). Lipases Novozym-435 (*Candida antarctica*, immobilized) and Lipozyme IM (*Mucor miehei*, immobilized) were gifts from Novo Nordisk Bioindustrials, Inc. (specified activity 7000 PLU/g and BAUN/g, respectively).

Immobilization of Lipase on Celite-521. The immobilization technique used for adsorption of the lipase on Celite was described elsewhere.[29] In summary, Celite-521 (2 g) was washed with distilled water and 0.1 M phosphate buffer (pH 7.0) and then added with gentle agitation to a solution of the lipase PS-30 (2 g) in 20 mL of 0.1 M phosphate buffer (pH 7.0). The resulting suspension was stirred for 2 h and then spread over a petri dish, and the water was removed under ambient conditions with occasional mixing. The activity of the enzyme determined by *p*NPA methanolysis (see below) was found to be 23 nmol of *p*NP min^{-1} mg^{-1}. The lipase preparation was then stored in a refrigerator at −10 °C prior to use.

Activity Assay of the Lipase PS-30. The activity of the immobilized and nonimmobilized form of lipase PS-30 was also measured by deacetylation of *p*-nitrophenyl acetate (*p*NPA, 1.45 mmol/L) at 37 °C to *p*-nitrophenol (*p*NP). The assay was conducted in anhydrous dioxane containing methanol (2.82 mmol/L) as described previously.[17] The activity reported as nanomoles of *p*NP formed per unit weight of enzyme per minute (nmol of *p*NP min^{-1} mg^{-1}) was found to be 23 and 10 for the immobilized and nonimmobilized form of the lipase.

Enzyme-Catalyzed Ring-Opening Polymerization of PDL. All reactions were carried out in bulk. The monomer (200 mg, 0.83 mmol) and lipase (50 mg of NI-PS-30 and 100 mg of I-PS-30) were separately dried in 6 mL reaction vials. In all cases, the monomer was dried over P_2O_5 in a desiccator (0.1 mm Hg; 38 h; room temperature). Three levels of reaction water content were obtained by drying the enzyme by one of the following methods: (*1*) the enzyme was used without drying, (*2*) the enzyme was dried over P_2O_5 in a desiccator (0.1 mmHg; 38 h; room temperature), and (*3*) the enzyme was dried over P_2O_5 using a diffusion pump in a drying pistol (65 μmHg; 38 h; 56 °C). The contents of the vials were mixed under dry argon atmosphere in a glovebag and securely capped. The vials were further sealed with Teflon tape and placed in constant temperature oil baths at predetermined reaction temperatures and time periods. Control reactions were conducted as above except that enzyme was not added. The reactions were terminated by dissolving the residual monomer and polymer in chloroform and separating the insoluble enzyme by filtration using 10−15 μm glass-fritted filters. The insoluble materials were washed two to three times with 5 mL portions of fresh $CHCl_3$, the filtrates were combined and solvent removed by rotary evaporation at 40 °C. Characterization of poly(PDL) (−[−O═C−CH_2^b−CH_2^c−{−CH_2^d−CH_2^d−}$_5$

Table 1. Polymerization of ω-Pentadecalalactone in Bulk at 80 °C Catalyzed by Lipases from Different Sources[a]

enzyme[b]	time (h)	conversion,[c] %	M_n[d]	M_w/M_n[d]
PS-30	24	85	24 000	2.4
PS-30	72	92	34 400	2.4
lipaseAK	24	98	17 600	2.4
lipaseAK	72	98	15 000	3.0
MAP-10	24	0		
MAP-10	72	<8		
Lipozyme IM[e]	24	80	26 900	2.2
Lipozyme-IM[e]	72	92	27 800	2.4
Novozym-435[e]	24	100	15 300	4.4
Novozym-435[e]	72	100	22 100	3.3

[a] Enzyme /monomer ratio in the reaction was 4/1 by weight. [b] The enzymes were dried according to method 2; see Experimental Section. [c] Calculated from ^{1}H-NMR spectra of the reaction mixture. [d] Determined by GPC. [e] The lipases Lipozym-IM and Novozym-435 were commercially available as immobilized preparations.

$-CH_2^c-CH_2^a-O-]-$) for a polymerization with quantitative conversion of monomer to polymer (M_n = 58 900) was as follows: ^{1}H-NMR ($CDCl_3$) 4.07 (protons *a*, 2H, t, J 6.7 Hz), 2.31 (protons *b*, 2H, t, J 7.5 Hz), 1.65 (protons *c*, 4H, m), and 1.30 (protons *d*, 22H, br s) ppm; ^{13}C-NMR ($CDCl_3$) 173.9, 64.4, 34.4, 29.6−29.1, 28.6, 25.9, and 25.0 ppm.

Instrumental Methods. ^{1}H-NMR spectra were recorded on a Bruker ARX-250 spectrometer at 250 MHz. Chemical shifts (in parts per million, ppm) were reported downfield from 0.00 ppm using trimethylsilane (TMS) as internal standard. The concentrations used were ~4% w/v in deuteriated chloroform ($CDCl_3$). The instrument parameters were as follows: temperature 300 K, pulse width 4.9 μs (30°), 32K data points, 3.17 s acquisition time, 1 s relaxation delay, and 32 transients. Monomer conversions were determined from the relative peak areas of signals corresponding to the ester methylene ($-C\underline{H}_2-O-C=O$) of the polymer (t, 4.05 ppm) and the ester methylene ($-C\underline{H}_2-O-C=O$) of the monomer (t, 4.15 ppm). For products of low number average molecular weight (<7200), molecular weights reported were calculated by ^{1}H-NMR from the relative areas of signals at 4.05 (see above) and 3.65 ppm (t). The signals at 3.65 ppm were due to chain terminal methylene ($-C\underline{H}_2-O-H$) groups. ^{13}C-NMR spectra were recorded at 62.9 MHz on a Bruker ARX-250 spectrometer. Chemical shifts (in parts per million, ppm) were referenced relative to $CDCl_3$ at 77.00 ppm.

Molecular weights >7200 were measured by gel permeation chromatography (GPC) using a Waters HPLC system equipped with a Model 510 HPLC pump, Waters Model 717 autosampler, Waters Model 410 refractive index detector (RI), Viscotek Model T60 viscosity detector and 500, 10^3, 10^4, and 10^5 Å ultrastyragel columns in series. Chloroform (HPLC grade) was used as eluent at a flow rate of 1.0 mL/min. The sample concentration and injection volumes were 0.5% (w/v) and 100 μL, respectively. Molecular weights were determined based on a universal calibration curve generated by narrow molecular weight distribution polystyrene standards (3.00×10^2, 1.00×10^3, 2.50×10^3, 4.00×10^3, 1.40×10^4, 9.00×10^4, and 2.07×10^5, Polysciences). RI and viscosity data were processed by Viscotek trisec GPC Software (version 3). The refractive index increment (dn/dc= 0.0285 at λ=632.8 nm in $CHCl_3$) was determined by a Wyatt/optilab 903 interferometric refractrometer (Wyatt Technology Inc.).

Total reaction water contents (wt % water) were measured by using a Mettler DL 18 Karl Fischer titrator with Hydranal-Titrant 5 (Fisher Scientific) and Hydranal Solvent (Fisher Scientific). The water w/w in reaction mixtures was determined by stirring 50 mg of enzyme and 200 mg of monomer in 2.5 mL of anhydrous DMSO for 24 h in a closed septum vial under argon atmosphere and then analyzing the water content of the supernatent relative to a DMSO control.

Results and Discussion

Screening of Enzymes. Screening results using selected commercial lipases for the bulk polymerization of PDL were given in Table 1. The reactions were carried out at 80 °C in bulk for 24 and 72 h. In all cases, the enzymes were dried by method 2 (at 0.1 mmHg, see Experimental Section). Except for lipase MAP-10, all of the other lipases studied were effective for PDL polymerization. Polymerizations catalyzed by lipases PS-30, AK, Lipozyme-IM and Novozym-435 gave % PDL conversions ranging from 80 to 100% for 24 h reactions. Furthermore, poly(PDL) M_n values ranged from 15 000 to 34 400. Based on these results, lipases PS-30, AK, MAP-10, Lipozyme-IM, and Novozym-435 all deserve further study. Since the initial results with PS-30 gave the highest molecular weight polymer as well as high conversion, this enzyme was selected for further study herein. It is important to note that no polymerization was observed for a control reaction conducted at 80 °C in the absence of lipase.

Poly(PDL) End Group Structure. The end-group structure of poly(PDL) was analyzed by selecting a product that had a relatively low M_n (7200) which was prepared by using PS-30 (5.6% water content, see below). The ^{1}H-NMR spectrum (not shown) of this product recorded at 250 MHz (see Experimental Section) showed signals at 3.65 (t) which were assigned to chain terminal methylene ($-C\underline{H}_2-O-H$) groups.[20,21] Furthermore, the ^{13}C-NMR spectrum (not shown) of this product recorded at 62.5 MHz (see Experimental Section) had a weak signal at 177.5 ppm which was due to the carbonyl of chain end carboxylic acids. This was confirmed by converting chain end carboxylic acids to their corresponding methyl ester (by using diazomethane) which caused the disappearance of this signal. No other chain end structures were observed for this product. Therefore, it was concluded that chains have terminal structure which consists of a carboxylic acid at one end and a hydroxyl at the other end. This is consistent with work presented elsewhere for lipase-catalyzed lactone ring-opening polymerizations for other monomer−enzyme pairs.[7,10,13,20,21] Previously it was hypothesized that water reacts with the enzyme-activated-monomer (EAM) complex[13,18] to form the corresponding hydroxy-acid monoadduct. This monoadduct will then react with EAM during propagation reactions to form higher chain lengths.[13,18] Considering the chain end structure of poly(PDL), it is likely that a similar mechanism is operative. Thus, the enzyme water concentration of PS-30-catalyzed PDL polymerizations may be a critical reaction parameter which regulates total chain number (see Discussion below).

Effect of Enzyme Immobilization. The rational for enzyme immobilization was described above (see Introduction). In this study, we compared immobilized (I) and nonimmobilized (NI) forms of the lipase PS-30 as catalysts for PDL polymerization. Immobilization of PS-30 was carried out by physical adsorption onto Celite-521 (see Experimental Section). Lipase preparations with different lipase to support ratios were prepared, and activities of the immobilized lipase were measured by the *p*NPA method (see Experimental Section). It was found that the preparation having a 1:1 (*w*/*w*) lipase/Celite ratio was the most active. Therefore, the 1:1 (*w*/*w*) ratio of PS-30/Celite was used for all subsequent PDL polymerizations with I-PS-30. Figure 1 shows that for 20 min and 3 h reaction times (70 °C, bulk), the I-PS-30 (dried by method 3; see Experimental Section) catalyzed polymerizations had % conversions of 26 and 63%, respectively. In contrast, NI-PS-30 (dried by method 3; see Experimental Section) catalyzed

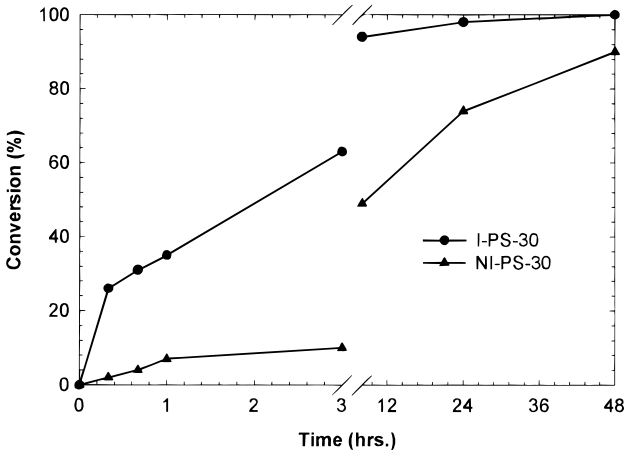


Figure 1. Monomer conversion as a function of time for the bulk polymerization of PDL catalyzed by NI- and I-PS 30 at 70 °C (reaction water content of 0.20% w/w).

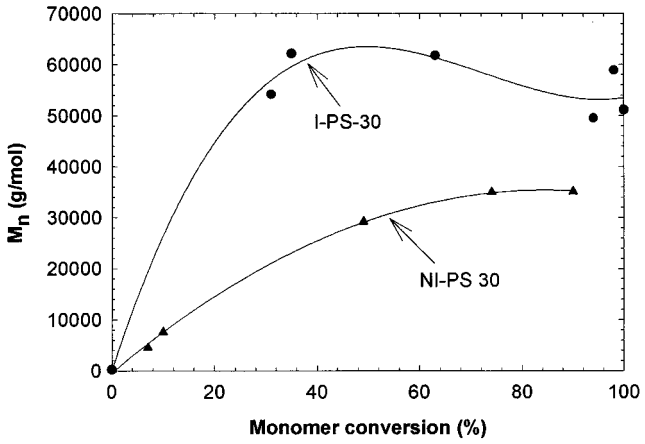


Figure 2. Number average molecular weight as a function of % conversion for the bulk polymerization of PDL catalyzed by NI- and I-PS 30 at 70 °C (reaction water content of 0.20% w/w).

reactions at 20 min and 3 h had % conversions of 2 and 10%, respectively. Therefore, for short reaction times, % conversions were more than 10 times greater by using the I-PS-30 catalyst. Nearly quantitative % conversions (>98%) were obtained by 8 h with I-PS-30 where, for similar % conversions, reactions catalyzed by NI-PS-30 required 48 h. Recovered I-PS-30 from a 48 h PDL polymerization was used to catalyze a second PDL polymerization. Comparison of the % conversion vs time results for the virgin and recovered I-PS-30 enzyme preparations gave almost identical results (data not shown). The increased activity of the lipase by adsorption on Celite might well be due to increased lipase surface area, as was suggested elsewhere.[28] However, further increase in the surface area of PS-30 by using a PS-30/Celite ratio of 1:4 (*w*/*w*) did not result in increased lipase activity based on *p*NPA methanolysis (see Experimental Section). Thus, it was concluded that surface area alone does not explain the magnitude of increased lipase activity which resulted by immobilization. Moreover, adsorption of porcine pancreatic lipase (PPL) onto Celite performed as was described herein for PS-30 did not result in enhanced activity for *p*NPA methanolysis. An alternative explanation is that adsorption of proteins onto surfaces may involve some degree of conformational change.[31−33] Immobilization of lipase PS-30 on Celite may change the enzyme conformation or orientation, which might lead to increased activity of molecules at the surface of immobilized particles. In other words, a change in enzyme conformation or orientation might allow enhanced access of substrate molecules to the lipase active site. However, this hypothesis has not been tested by experimental investigations.

The variation in poly(PDL) molecular weight vs % conversion shown in Figure 2 (70 °C, bulk) was studied for the same set of experiments described above for Figure 1. Since the monomer and enzyme for these reactions were dried under stringent conditions (see above), the % water present in reactions was low (0.20% w/w for I- and NI-PS-30). Under these conditions, the use of I-PS-30 as opposed to NI-PS-30 at comparable % conversions gave substantially higher product M_n values. Also, by 35% conversion (1 h reaction time), the product from I-PS-30 catalysis had an M_n of 62 000. Further increase in the % conversion for I-PS-30 catalyzed polymerizations resulted in no substantial changes in poly(PDL) M_n. Polymerizations catalyzed by NI-PS-30 showed a steady increase in M_n up to 49% conversion, after which, M_n showed little change. Polydispersities (M_w/M_n) for products formed by both NI- and I-PS-30 catalysis ranged from 1.8 to 2.0 and showed little change with conversion. The formation of high molecular weight chains by about 40% conversion is consistent with chain polymerizations where the rate of propagation is much faster than initiation. That poly(PDL) M_n showed little or no change with increases in % conversion above about 40% may result from decreased propagation rates of preformed chains. Increasing conversion may result from the formation of new propagating chains by reactions between the EAM complex and water.

In reviewing studies by Kobayashi and co-workers[21] of DDL polymerization at temperatures between 60 and 75 °C, M_n increased with conversion for lipases PF, CC (from *C. cylindracea*), PC (from *P. cepacia*), and PPL but remained unchanged with conversion (41−99%) for immobilized lipase PS (from *Pseudomonas* sp.). Furthermore, product polydispersities of poly(DDL) prepared at 75 °C by different enzymes ranged from 2.4 to 4.3.[21] At present, we do not have a fundamental understanding of why different enzyme catalysts result in different product molecular weights and dispersities. Some factors which may be important in molecular weight regulation will be discussed below. It is noteworthy to point out that prior to this publication, the highest M_n reported for enzyme-catalyzed lactone ring-opening polymerization was 25 000 (immobilized lipase PS-catalyzed DDL polymerization).[21]

Effect of Reaction Temperature for I-PS-30-Catalyzed Polymerizations. Kobayashi and co-workers found large increases in % DDL conversion by increasing the reaction temperature of NI-lipase PF (from *P. Fluorescens*) and lipase CC-catalyzed polymerizations. However, reaction temperatures above 75 °C were not investigated.[21] Polymerization reactions catalyzed by I-PS-30 were performed at 60, 70, 80, 90, 100, and 110 °C. No polymerization was observed for a control reaction conducted at 110 °C in the absence of lipase. For these experiments, the enzyme was dried by method 2 (see Experimental Section) so that the % water present in reactions was intermediate (0.30% w/w, see below). The effect of reaction temperature on % monomer conversion was shown in Figure 3. Three sets of experiments were performed where the reaction time was kept constant at either 15 min, 1 h, or 8 h and the

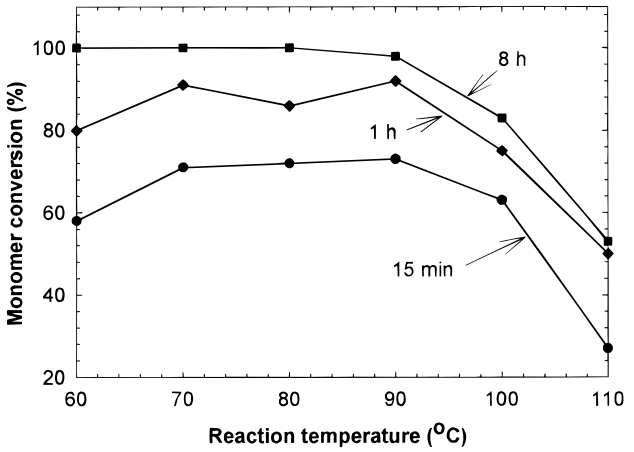


Figure 3. Effect of reaction temperature on % monomer conversion for I-PS-30 catalyzed polymerizations conducted for 15 min, 1 h and 8 h (reaction water content of 0.30% w/w).

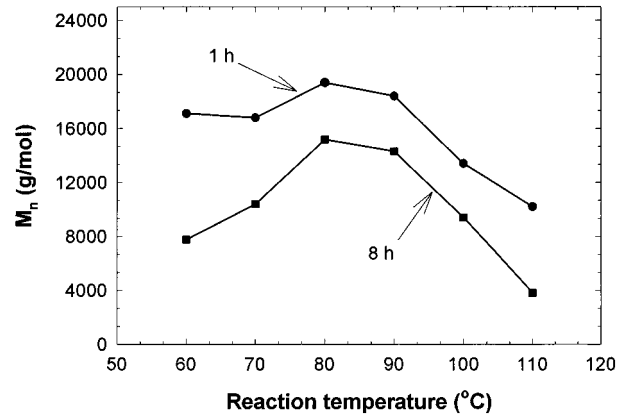


Figure 4. Effect of reaction temperature on poly(PDL) number average molecular weight for I-PS-30 catalyzed polymerizations conducted for 1 and 8 h (reaction water content of 0.30% w/w).

temperature was varied. The general trends observed by comparison of these experiments were as follows: (*i*) increased % conversion by increasing the reaction temperature from 60 to 70 °C, (*ii*) similar polymerization rates between 70 and 90 °C, (*iii*) a small decrease in monomer conversion (by ∼10%) as the reaction temperature was increased from 90 to 100 °C, and (*iv*) a large decrease in monomer conversion (by ∼30%) as the reaction temperature was increased from 100 to 110 °C. Therefore, the I-PS-30 catalyst was an effective catalyst for the conversion of PDL to poly(PDL) at temperatures between 90 and 100 °C. The high thermal stability of I-PS-30 may be important for reactions where high reaction temperature conditions are required.

Figure 4 showed that the reaction temperature was a critical parameter which regulated poly(PDL) molecular weight. The similarity of % conversion values between 70 and 100 °C for a fixed reaction time made it possible to directly compare changes in M_n without correcting for effects of % conversion. The following general trends in M_n were observed: (*i*) the molecular weight decreased substantially at all temperatures as the polymerization time was extended from 1 to 8 h, (*ii*) an increase in the reaction temperature from 70 to 80 °C resulted in larger M_n values, (*iii*) an increase in the reaction temperature from 80 to 90 °C did not change M_n, (*iv*) along with % conversion, M_n also decreased substantially when the reaction temperature was increased from 100 to 110 °C. Thus, the highest poly(PDL) molecular weights were observed by conducting polymerizations between 80 and 90 °C. In studies by Kobayashi for DDL lipase-catalyzed polymerizations, increasing the reaction temperature between 30 and 75 °C also led to increased product M_n.[21] Comparison of the results in Figure 4 to those given in Figure 2 suggests that poly(PDL) M_n values >62 000 may result by carrying out polymerizations at between 80 and 90 °C with low (<0.20%) reaction water content. In other words, to achieve optimal molecular weights for poly(PDL) polymerizations catalyzed by I-PS-30, we believe that the reaction water content and polymerization temperature must be carefully adjusted. A systematic investigation of the reaction water content is described below.

The formation of the largest poly(PDL) molecular weights at temperatures between 80 and 90 °C may be due to several factors. We are in agreement with Kobayashi and co-workers that an important effect of increased reaction temperature is monomer and polymer diffusion.[21] Therefore, as the polymerization proceeds, there is an increase in the viscosity of the system whereby access of the propagating chain end to monomer becomes diffusion-controlled. Thus, as the polymerization temperature was increased from 60 to between 80 and 90 °C, the diffusivity of poly(PDL) chains increased, leading to higher molecular weight products. In other words, by increasing the polymerization temperature, chains may continue to grow at relatively higher molecular weights prior to reaching an upper limit where diffusion constrains propagation. Another explanation is that lipase active sites may have low reactivity for chains that exceed certain molecular weight values. Furthermore, variation in temperature may change enzyme and polymer substrate conformation such that increased chain lengths are suitable substrates for propagation as the reaction temperature was increased to between 80 and 90 °C. Decreases in product M_n as the reaction temperature was increased above 90 °C may be explained by the following: (*i*) enzyme denaturation which decreases polymerization rates, (*ii*) increased importance of enzyme-catalyzed chain degradation reactions such as hydrolysis and (*iii*) an increase in chain initiation reactions which would give higher total chains and lower M_n, and (*iv*) an increase in chain depolymerization due to backbiting reactions.

Effect of Water Content. The role of water in chain initiation was discussed above and elsewhere.[13,18] Water content for NI-PS-30- and I-PS-30-catalyzed reactions conducted at 70 °C was varied by using the three different drying methods described in the Experimental Section. The effect of water content on the % monomer conversion for NI-PS-30- and I-PS-30-catalyzed reactions were shown in parts a and b of Figure 5, respectively. Inspection of the results showed that, with an increase in the water content in reactions, enhanced polymerization rates were achieved. For example, for NI-PS-30, % monomer conversions were 67 and 10% at 3 h reaction times for 5.60 and 0.20% w/w water, respectively. Furthermore, for I-PS-30-catalyzed reactions conducted for 1 h, increasing the water content from 0.20 to 0.50% w/w resulted in an increase in the % monomer conversion from 35 to 95%. One factor which may be responsible for the above is that by increasing the reaction water content there is a corresponding increase in chain initiation and, therefore, an increase in the number of propagation chain ends in the

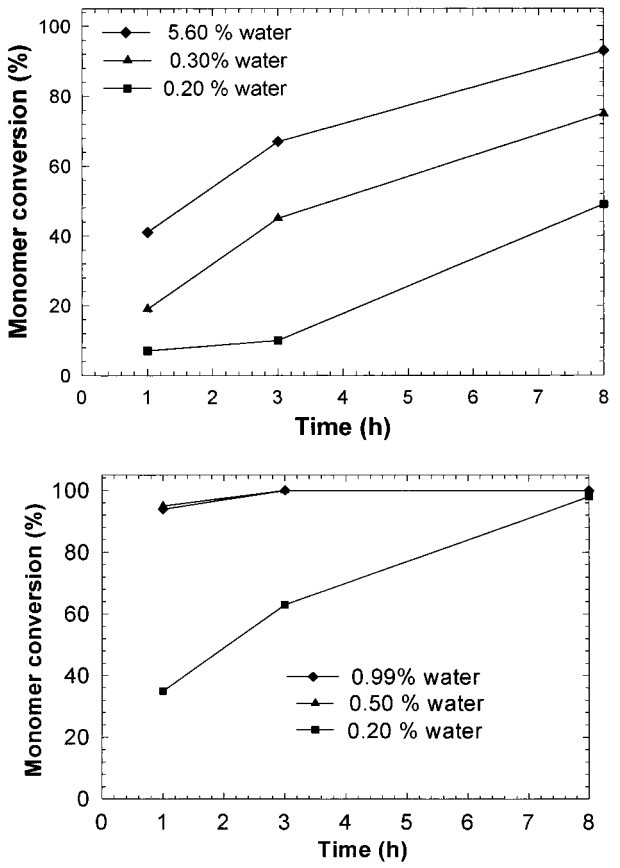


Figure 5. Variation in %-monomer conversion with time at different reaction water contents for (a) NI-PS-30- and (b) I-PS-30-catalyzed PDL polymerizations at 70 °C.

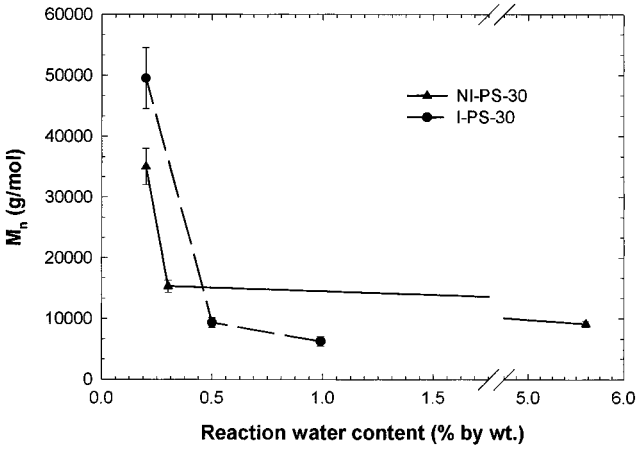


Figure 6. Variation in poly(PDL) molecular weight as a function of the reaction water content (percent by weight) for polymerizations conducted for 24 h at 70 °C. Error bars give the standard deviation for $n = 3$.

reaction. An increase in the number of propagating chains may result in increased polymerization rates. In addition, variation of water content in organic media is known to alter enzyme activity.[1,34] Thus, it may be that PS-30 activity was enhanced by increasing the shell of enzyme-bound water.

Water content in reactions was also found to be important in regulating product molecular weight. The effects of reaction water content on M_n were shown in Figure 6 for reactions conducted for 24 h at 70 °C. For NI-PS-30, M_n increased from 9070 to 35 000 by decreasing the reaction water content from 5.60 to 0.20%. I-PS-30-catalyzed PDL polymerizations conducted at 0.99% water content gave low molecular weight poly(PDL) (M_n = 6200). However, by lowering the water content to 0.20%, the product M_n was 49 500. Interestingly, decreasing the reaction water content for I-PS-30 from 0.99 to 0.50% resulted in a small increase in molecular weight (6200 to 9300) relative to that observed when the water content was further decreased to 0.20% (see Figure 6). Similarly, decreasing the water content from 5.60 to 0.30% for NI-PS-30-catalyzed polymerizations resulted in a relatively small increase in molecular weight (9100−15 300) compared to that observed by further decreasing the water content to 0.20% (see Figure 6). The decrease in poly(PDL) molecular weight with increasing reaction water content might be a consequence of an increase in the total chain number (see above). Indeed, in previous work by our laboratories for PPL-catalyzed ϵ-CL polymerization, we showed that M_n could be predicted from [M]/[I] where [M] and [I] are the monomer and initiator concentrations, respectively.[13,18] Furthermore, we showed that [I] was an additive function of the total nucleophile concentration. Moreover, when water was the sole nucleophile, only a fraction of the water present reacted to initiate chains.[18] However, as the quantity of water in polymerization reactions increased, the total water which reacted to initiate chains also increased.[13] It is indeed possible that by further drying the enzyme using a different protocol than drying method 3 (see Experimental Section), significantly higher product molecular weights may result. However, as was shown above, the increase in molecular weight obtained by decreasing the reaction water content would be accompanied by a corresponding decrease in the polymerization rate. Future work will address the need to achieve high product molecular weight without sacrificing rapid monomer polymerization.

Summary of Results

Relative to other lipases screened, we found that PS-30 gave the highest molecular weight and high monomer conversion. Hence, PS-30 was selected for further investigations. Comparisons of bulk PDL polymerization at 70 °C catalyzed by NI-PS-30 and I-PS-30 (dried by method 3) showed that for short reaction times (≤20 min), % conversions were more than 10 times greater by using the I-PS-30 catalyst. Furthermore, for I-PS-30- and NI-PS-30-catalyzed reactions under these conditions, the immobilized catalyst gave product of substantially higher molecular weight. Interestingly, when % conversion was increased above ∼40%, there was little or no change in PDL M_n. This is consistent with chain polymerizations where the rate of propagation is much faster than initiation. Competing reactions such as chain cleavage by enzyme-catalyzed hydrolysis were not observed for these polymerizations where reaction water levels were low. The increased activity of the lipase by adsorption onto Celite may in part result from increased lipase surface area. Alternatively, enzyme immobilization may alter enzyme conformation or orientation and thereby enhance the accessibility of substrate molecules to the lipase active site.

The I-PS-30-catalyzed polymerizations of PDL were also studied as a function of reaction temperature. We observed that the % conversions were similar between 70 and 90 °C. However, M_n values were largest for polymerization temperatures between 80 and 90 °C. Further increase in the polymerization temperature above 100 °C resulted in a lower % conversion and M_n.

Studies were also carried out to determine the effect of reaction water content on % conversion and poly(PDL)

molecular weight. An increase in the water content in reactions allowed enhanced polymerization rates to be achieved. For example, for I-PS-30-catalyzed reactions conducted for 1 h, increasing the water content from 0.20 to 0.50% w/w resulted in an increase in the % monomer conversion from 35 to 95%. This effect of water may result from an increase in the number of propagating chains. Also, variation of the water content may enhance enzyme activity. Measurements of molecular weight showed that poly(PDL) M_n was inversely proportional to the reaction water content. We concluded that, to achieve optimal poly(PDL) molecular weights, there must be careful control of both the polymerization temperature and reaction water content. Elevated polymerization temperatures appear important to overcome limitations imposed by chain diffusion in bulk polymerizations. At low reaction water levels (0.20% w/w water), the I-PS-30-catalyzed polymerization of PDL at 70 °C gave poly(PDL) with an M_n and M_w/M_n of 62 000 and 1.9, respectively. Thus far, this is the highest molecular weight polyester prepared by an enzyme-catalyzed polymerization reaction.

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