Enzyme-Catalyzed Polymerizations of ϵ-Caprolactone: Effects of Initiator on Product Structure, Propagation Kinetics, and Mechanism

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ABSTRACT: Studies were undertaken to gain mechanistic information on lactone ring-opening polymerization reactions using porcine pancreatic lipase (PPL) as the catalyst and ϵ-caprolactone (ϵ-CL) as the monomer. Polymerizations were carried out at low water levels (0.13 mmol) and supplemented with either butanol or butylamine. Rates of monomer conversion, product molecular weight, total chain number, and chain end structure were determined by ^{1}H NMR. In the presence of water alone, a maximum M_n of 7600 g/mol was obtained at 85% conversion, which decreased to 4200 g/mol as the reaction continued to 98% conversion. Reactions with butanol and butylamine at 100% conversion gave polymers with M_n values of 1900 and 1200 g/mol, respectively. For these three polymerizations, the total number of polymer chains increased with conversion due to a simultaneous increase in carboxylic acid chain ends. Within 4 h (∼26% monomer conversion), butylamine was completely consumed but only 37% of butanol reacted. Reactions with butylamine occurred predominantly by an enzyme-mediated route to form *N*-butyl-6-hydroxyhexanamide. This step was rapid relative to subsequent chain growth. In addition, the living or immortal nature of the polymerizations was assessed from plots of $\log\{[M]_0/[M]_t\}$ versus time and M_n versus conversion. These results indicate that termination and chain transfer did not occur, and we described the system as providing "controlled" polymerizations. Furthermore, an expression for the rate of propagation was derived from the experimental data which is consistent with that derived from the proposed enzyme-catalyzed polymerization mechanism. The absence of termination in conjunction with the relationship between molecular weight and the total concentration of multiple initiators suggests that ϵ-CL polymerization by PPL catalysis shares many features of immortal polymerizations.

Introduction

Investigation into enzyme-catalyzed polyester synthesis by using suspensions of commercial lipases in essentially anhydrous organic solvents has received increased attention over the past few years. A variety of polymers were synthesized from the ring-opening of 6-, 7-, 12-, 13-, and 16-member lactones. Initially, Knani *et al.*[1] successfully synthesized poly(ϵ-caprolactone) (PCL) using porcine pancreatic lipase and studied the effects of methanol upon initiation. Uyama and Kobayashi[2] found the lipase from *Pseudomonas fluorescens* to be a preferred catalyst for bulk ϵ-CL polymerization (initiating with water). MacDonald *et al.*[3] studied PPL-catalyzed ϵ-CL polymerization and demonstrated the effects of nucleophiles on modulating chain end structure as well as how polymer molecular weight varied with changes in the monomer to initiator molar ratio. Uyama *et al.*[4] showed that at least for selected lipases, lipase-catalyzed polymerization of the macrolides 11-undecanolide and 15-pentadecanolide resulted in higher molecular weights and faster rates of polymerization relative to polymerizations of ϵ-CL.

In general, enzyme-catalyzed polymerizations require long reaction times for complete monomer conversion and have resulted in low number-average molecular weight (M_n) polyesters. In addition, the polymerization steps normally proceed with low rates of propagation. Thus, our goal herein was to obtain a better understanding of enzyme-catalyzed polymerization mechanistic features to increase propagation kinetics and decrease pathways which limit product molecular weight. Presently, information on the mechanism for enzyme-catalyzed polyester synthesis by lactone ring-opening is limited. For example, Kobayashi and co-workers[5] reported that lipase-catalyzed lactone polymerization using water as the sole initiator proceeded via esterification between two molecules of ω-hydroxycarboxylic acid, transesterification of this product with a lactone monomer, or competitively by these two routes. In contrast, MacDonald *et al.*[3] reported that PPL-catalyzed ϵ-CL polymerization in heptane (65 °C) occurs predominantly by ring-opening. The mechanism proposed involved the reaction of ϵ-CL with a serine residue of PPL at the catalytically active site to form an acyl-enzyme activated monomer which then reacted with the propagating chain end. The slow increase in PCL molecular weight with conversion indicated that this was a chain polymerization with slow propagation.[3] Later, work by Uyama *et al.*[4] also concluded that lactone polymerization occurred by the above-described enzyme-activated monomer mechanism.

Assuming that ϵ-CL reacts with the lipase to form a stable propagating species, the nature of the chemical intermediate formed is very important. It is generally

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believed that similar acyl-enzyme intermediates are covalently linked.[6,7] The existence of covalent propagating centers as in some cationic polymerizations[8] raises the question of its reactivity. In this paper, the possibility that the acyl-enzyme intermediate has a reactivity which permits slow propagation but not chain termination is considered. An interesting model is that described by Inoue for the "immortal" polymerization of ϵ-CL initiated by an aluminum porphyrin catalyst.[9] Inoue showed that these porphyrinato-catalyzed nucleophilic reactions have rates of initiation that are faster than propagation. Polymers of relatively high molecular weight (7400 g/mol at 100% monomer conversion) with narrow molecular weight distributions (1.07−1.13) were formed because chain termination did not occur even in the presence of protic substances. The total number of chains was additive with respect to the initiator and other protic nucleophiles.

In this study, ϵ-CL ring-opening polymerization reactions using PPL as the catalyst were investigated. Polymerizations were carried out at low water levels using different monomer to butanol or butylamine ratios with reaction times from 0.5 to 96 h. The resulting polymers were analyzed by ^{1}H nuclear magnetic resonance to determine monomer conversion, relative rates of initiation, total number of chains, and chain end structure. These heterogeneous enzyme-catalyzed polymerizations were assessed by living critieria and compared to "immortal" polymerization systems. In addition, a rate expression for propagation was derived from plots of $\log\{[M]_0/[M]_t\}$ versus time and M_n versus conversion.

Experimental Section

Synthetic Procedure. Synthesis of Poly(ϵ-caprolactone). Polymerizations were carried out in the presence of heptane at 65 °C, varying the concentrations of either butanol or butylamine and measuring the water content. The enzyme used was porcine pancreatic lipase Type II Crude from Sigma Chemical Co. (~25% protein). Enzyme activity (465 units/mg of protein) was determined using a modified version of Procedure N800 provided by Sigma Diagnostics, which involves measuring the fatty acid liberated by lipase-catalyzed hydrolysis of olive oil after an incubation period of 3 h at 37 °C. In this work, the end point from titrating with sodium hydroxide was determined by changes in the solution pH using a Mettler DL18 pH meter. PPL was sieved with mesh screens to obtain a particle size between 60 and 80 mesh. The sieved enzyme (750 mg) was transferred into oven-dried vials and dried either in a vacuum desiccator (0.1 mmHg, 25 °C, 16 h) or by a diffusion pump equipped with a drying pistol apparatus (5−10 μmHg, 25 °C, 44 h). The dried enzyme was transferred under argon to oven-dried 50 mL Erlenmeyer reaction flasks, which were stoppered immediately with rubber septa and purged with argon. The subsequent reagents were added via syringe: dry heptane (5 mL/34.12 mmol, Aldrich, fractionally distilled over CaH_2 under argon), butanol (Aldrich, fractionally distilled over Na° under argon) or butylamine (Aldrich, distilled over CaH_2 under argon) and ϵ-CL (0.5 mL/4.51 mmol, Aldrich, fractionally distilled over CaH_2/1.7 mmHg, 83 °C). The reaction flasks were placed into a shaker incubator (65 °C, 200 rpm) for reaction times of 4−96 h unless otherwise specified. During the course of the polymerizations, the particulate enzyme was surrounded by swollen product and these gel-like particles agglomerated. The reactions were terminated by vacuum filtering the sample to separate the enzyme catalyst from the product and/or residual materials. The filtered enzyme and residual materials were subsequently washed with several portions of chloroform, and the filtrates were combined. To remove volatiles, including unreacted monomer, the solution was rotary evaporated (15 mmHg, 50−55 °C, 2 h) and then placed in a vacuum oven (2 mmHg, 65 °C, 48 h). The resulting products were analyzed by ^{1}H NMR and gel permeation chromatography (GPC). The conversion of ϵ-CL to PCL was determined as the product weight after vacuum oven drying relative to the initial weight of ϵ-CL charged to polymerization flasks. The water content of all reagents was measured by using a Mettler DL18 Karl Fischer titrator with Hydranal-Titrant 5 (Fisher Scientific) and Hydranal Solvent (Fisher Scientific). The concentration of water associated with the dried enzymes was determined by stirring 750 mg of enzyme in 10 mL of methanol (anhydrous grade) for 16 h and then analyzing the filtrate relative to a methanol control. The reported concentrations of water are the total from all reagents employed, including the enzyme.

Investigations of Reactions at Low Conversion. PPL was dried using the diffusion pump, and butanol, butylamine, and ϵ-CL were purified as described above. To an oven-dried 50 mL Erlenmeyer flask were added (under argon) PPL (750 mg), butanol (0.42 mL/4.6 mmol) or butylamine (0.42 mL/4.2 mmol), and ϵ-CL (7.00 mL/63.2 mmol). The reactions were then placed into a shaker incubator (65 °C, 200 rpm). Aliquots (0.50−0.60 mL) of the reaction mixture were removed via syringe every 0.5 h for a total of 2 h and filtered (terminating the reaction). The filtered materials were washed several times with 1.0 mL portions of chloroform-*d* ($CDCl_3$). The filtrates were then combined and diluted with $CDCl_3$ to prepare solutions (20 mg/mL) for ^{1}H NMR analysis. An experimental control was also carried out by omission of the enzyme catalyst from the reaction mixture containing butylamine and ϵ-CL.

Hydrolysis of PCL. Four polymerization reactions were set up using dried reagents as described above (see Synthesis of PCL) with a ratio of ϵ-CL to butylamine of 15/1. After 96 h, one reaction was terminated for analysis while either 0, 0.56, or 1.10 mmol of water was added to the remaining flasks. After a total of 139 h, the reactions were terminated by filtration and the PCL was isolated as described above.

Instrumental Procedures. A Varian UNITY-300 NMR spectrometer was used for all NMR experiments described herein. ^{1}H NMR spectra were recorded at 300 MHz. Chemical shifts in parts per million (ppm) were reported downfield from 0.00 ppm using tetramethylsilane (TMS) as the internal reference. The experimental parameters were as follows: 2.0% (w/v) polymer in chloroform-*d*, temperature 298 K, 14 μs pulse width, 3.7 s acquisition time, 16 repetitions, and 3000 Hz spectral width. The following characterizations of polymer products were determined by ^{1}H NMR: degree of polymerization and corresponding number-average molecular weight, mole fraction of butyl ester and carboxylic acid end groups, total number of polymer chains, and monomer conversion. Details explaining how this information was obtained from NMR spectra followed exactly as was described previously.[3] Modifications to the above for determinations of butyl amide end groups and butylamine reactivity is described below (see Results and Discussion).

Molecular weights were also measured by GPC using a Waters Model 510 pump, Model 410 refractive index detector, and Model 730 data module with 500, 10^3, 10^4, and 10^5 Å Ultrastyragel columns in series. Chloroform (HPLC grade) was used as an eluent at a flow rate of 1.0 mL/min. The sample concentrations and injection volumes were 0.5% (w/v) and 100 μL, respectively. Polystyrene standards with molecular weights of 3.00×10^2 , 1.00×10^3, 2.50×10^3, 4.00×10^3, 2.40×10^4, 9.00×10^4, and 2.07×10^5 (Polysciences) were used to generate a calibration curve. The integration values were defined to include all retention times prior to that of ϵ-CL.

Results and Discussion

Characterization of Polymer Products by ^{1}H NMR Spectroscopy. Interpretation of product ^{1}H NMR spectra for water and butanol (BuOH) initiated polymerizations were described previously.[3] The ^{1}H NMR spectrum for a polymerization carried out for 96 h using butylamine, ($BuNH_2$), as an initiator is shown in Figure 1. In addition to previous spectral assign-

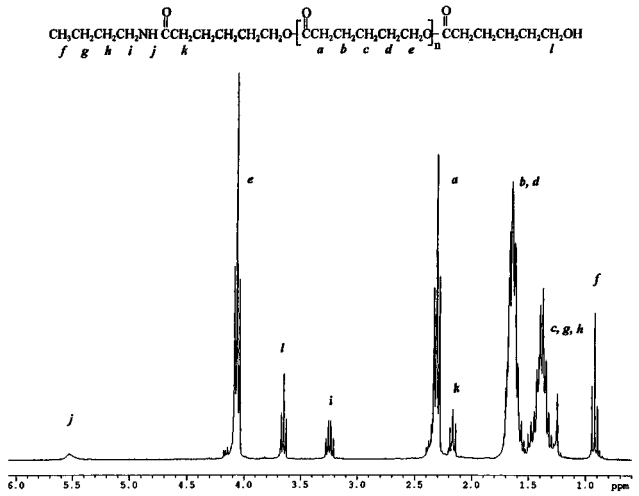


Figure 1. ^{1}H NMR (300 MHz) spectrum recorded in chloroform-*d* of the product obtained from the ring-opening polymerization of ϵ-CL using butylamine (ϵ-CL/$BuNH_2$ = 15/1) as the initiator after a 96 h reaction time.

ments,[3] signals centered at 5.55 (broad singlet), 3.24 (quartet), and 2.18 (triplet) ppm were assigned to protons j, i and k, respectively (see Figure 1). These assignments were made based on chemical shift parameters for model compounds[10] and were confirmed by ^{1}H−^{1}H correlations determined by recording a homonuclear proton 2D NMR spectrum (COSY, spectrum not shown). The degree of polymerization (X_n) and the corresponding number-average molecular weight values of the polymers obtained from butylamine were measured by comparing the spectral integration intensities of the methylene protons adjacent to the oxygen of intrachain repeat units at 4.05 ppm (protons e) and terminal hydroxyl groups at 3.65ppm (protons l). The mole fraction of butyl amide end groups was determined by comparison of the spectral integration values of protons i and l (see Figure 1). The mole fraction of carboxylic acid end groups was determined by derivatization with diazomethane as described previously.[3]

Products Formed at Low Monomer Conversions. Experiments were conducted at early stages of the reaction (0−2 h) to provide information on the relative rates of initiation as a function of the nucleophile added as well as the initial products formed. The polymerizations were carried out in bulk at 65 °C and aliquots of the reaction mixture were withdrawn every 0.5 h (see Experimental Section, synthetic procedures, for additional details). ^{1}H NMR spectra of reaction mixtures after 2 h reaction times were recorded and are shown in Figure 2a,b. Assignments of protons f′−l′ for *N*-butyl-6-hydroxyhexanamide (see Figure 2a) were made by using chemical shift parameters of suitable model compounds[10] and by comparison to ^{1}H NMR spectra of corresponding polymer products (Figure 1). Assignments of chemical shifts for protons 1−5 of ϵ-CL were given elsewhere.[11] Interestingly, the spectrum in Figure 2a shows no apparent signal for protons a (Figure 1) but does show a signal corresponding to protons k′ at 2.18 ppm. This suggests that PCL oligomers were not formed at this stage of the reaction. Moreover, the integration values for the signals corresponding to k′ and l′ are approximately equal (within 3%), further indicating that no PCL oligomers were formed. Because no apparent signal was observed at 2.35 ppm assigned to the methylene protons adjacent to PCL carboxylic acid chain end groups,[2,3] it is concluded that up to 2 h, 6-hydroxycaproic acid was not

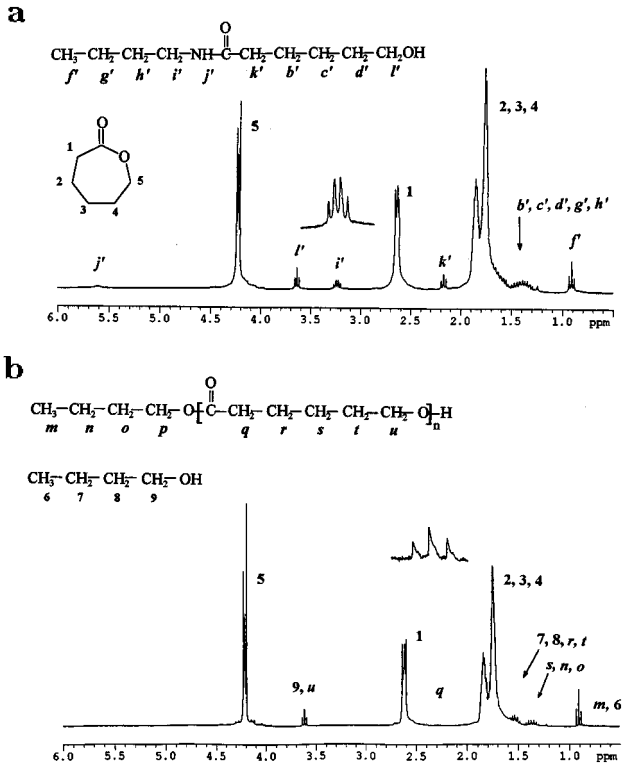


Figure 2. ^{1}H NMR (300 MHz) spectra recorded in chloroform-*d* of products at low percent conversion (2 h reaction time) using (a) ϵ-CL/$BuNH_2$ = 15/1 and (b) ϵ-CL/BuOH = 14/1. Expansions provided represent i′ and q, respectively.

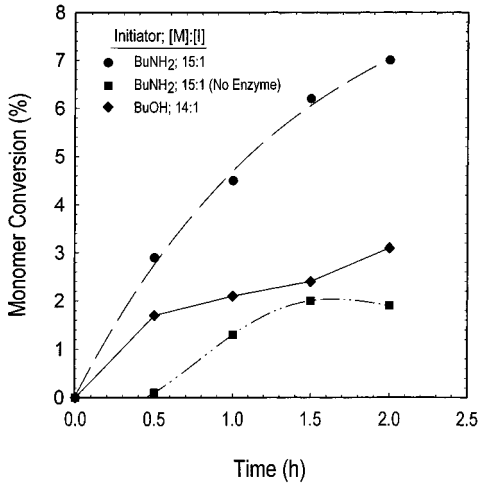


Figure 3. Monomer conversion at short reaction times.

formed. The monomer consumption as a function of time for reactions with butylamine (Figure 3) was determined by comparison of the signal intensities corresponding to protons i′ and 5 (see Figure 2a). Results for monomer consumption were also obtained for control reactions containing butylamine without addition of enzyme (see Figure 3). After a 0.5 h reaction time, the percent monomer converted for the control reaction was negligible (<0.1%) whereas 2.9% monomer was consumed with enzyme. At 2 h, monomer conversion for butylamine-initiated reactions without and with enzyme was 1.9 and 7.0%, respectively. The theoretical percent monomer conversion is 7.1% for a ϵ-CL/butylamine ratio of 15/1 assuming quantitative formation of *N*-butyl-6-hydroxyhexanamide. This value is in excellent agreement with that observed experimentally after 2 h. Thus, non-enzyme-mediated ring-opening of ϵ-CL by butylamine occurs but at a rate which is

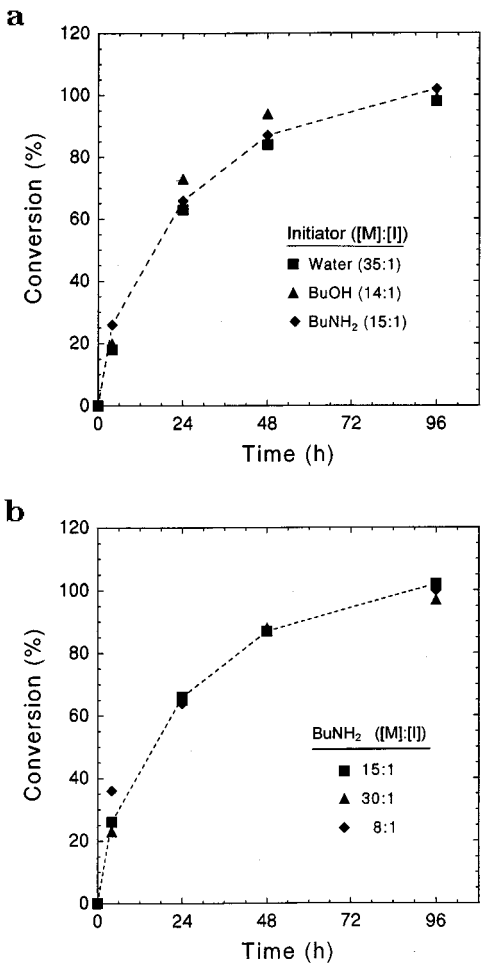


Figure 4. Monomer conversion as a function of time for polymerizations: (a) using butylamine, butanol, or only water as nucleophiles; (b) variable ratios of ϵ-CL to butylamine.

considerably slower than corresponding enzyme-catalyzed reactions. Furthermore, for reactions with enzyme, butylamine was consumed within 2 h and formed *N*-butyl-6-hydroxyhexanamide prior to subsequent chain growth.

The percent monomer conversion for reactions with butanol was determined by comparison of the spectral intensities of protons q at 2.30 ppm with protons 1 at 2.63 ppm. After 0.5 and 2 h, 1.7 and 3.1% of ϵ-CL was consumed (see Figure 3). Therefore, chain initiation by butylamine is more rapid than butanol. Based on previous work,[3] it was assumed that for butanol, non-enzyme-mediated reactions do not occur within this time frame. Unfortunately, since the chemical environments of protons a (see Figure 1) and q as well as protons 9 and u (see Figure 2b) are similar (resolution not achieved), it was not possible to determine ring-opened products at initial stages of this reaction.

Polymerization Reactions in Heptane. PPL-catalyzed ϵ-CL ring-opening polymerizations were carried out in heptane at 65 °C. In addition to the total water content of 0.13 mmol (see Experimental Section), polymerizations were supplemented with either butanol (0.33 mmol) or butylamine (0.30 mmol) as competitive nucleophiles for chain initiation. The corresponding molar ratios of ϵ-CL/water, ϵ-CL/butanol, and ϵ-CL/butylamine were 35/1, 14/1, and 15/1, respectively. Both conversion and M_n as a function of time were determined. Figure 4a shows that the curves generated for percent conversion versus time were similar for the

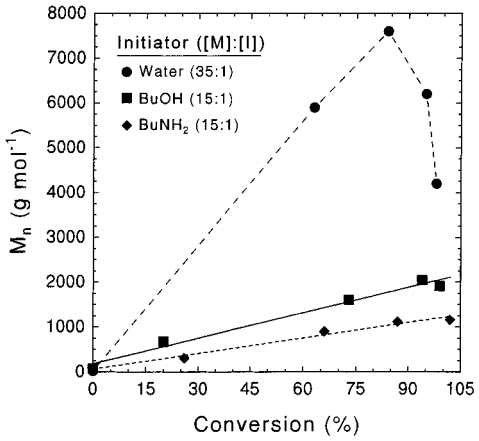


Figure 5. Product molecular weight (M_n) as a function of percent conversion for polymerizations using butylamine, butanol, or only water as nucleophiles.

three polymerizations. In each system, up to 96 h, we observe a monotonic increase in monomer conversion, implying that consumption is not zero order with respect to monomer concentration. Overall, the most notable difference observed was a slightly higher percent conversion value (26% relative to 19 and 17%; see Figure 4a) for reactions with butylamine at low conversion. This is explained by relatively faster chain initiation with the competitive nucleophile butylamine (see discussion above).

Figure 5 shows a plot of M_n versus percent conversion for the same three systems. The results varied considerably as a function of the initiator system used. For example, in the presence of water (0.13 mmol) without butanol or butylamine, a maximum M_n of 7600 g/mol was obtained at 85% conversion. The value, however, decreased to 4200 g/mol as the reaction continued to 98% conversion. The two reactions carried out with added butanol (0.33 mmol) and butylamine (0.30 mmol) at 100% conversion gave polymers with M_n values of 1900 and 1200 g/mol, respectively. It should be noted that for all three polymerizations, M_n either decreased substantially or stayed almost unchanged at high conversions (see Figure 5). This may be due to decreased chain propagation rates at high conversion so that competing reactions which decrease M_n such as chain cleavage by hydrolysis become increasingly important. M_n values determined by GPC (values not shown) as a function of time were similar to those measured by ^{1}H NMR (corresponding average deviation of 12%). In general, product polydispersity increased with percent conversion. For example, reactions with butanol and butylamine showed increased M_w/M_n values from 1.7 to 2.9 and 1.5 to 2.4 for percent conversions of 20 to 94 and 26 to 87, respectively. As these reactions approached 100% monomer conversion, the molecular weight distribution increased further to 3.6 and 3.4, respectively. Polymerizations carried out without butanol or butylamine had M_w/M_n values as high as 5.0 at 84% conversion. Factors which may contribute to broadening of molecular weight distributions at prolonged reaction times will be discussed below.

Figure 6a shows the relationship between total chain number and percent conversion. In Figure 6b, changes in the mole fraction of end group functionalities as a function of percent conversion are shown for polymerizations with ϵ-CL/BuOH and ϵ-CL/BuNH$_2$ ratios of 14/1 and 15/1, respectively. For all of the polymerizations,

Table 1. Product Analysis Obtained from Reactions with Water after Polymerization

product[a]	water added[b] (mmol)	conv[c] (%)	^{1}H NMR M_n (g/mol)	GPC M_w/M_n	mol fraction[d] COOH (BuNH)	butylamine reacted (mmol)	water reacted (mmol)	total chains[e] (mmol)
1	0	84	1150	3.2	0.27 (0.62)	0.23	0.10	0.37
2	0	82	1130	3.0	0.40 (0.57)	0.21	0.15	0.37
3	0.56	89	840	2.6	0.57 (0.45)	0.24	0.31	0.54
4	1.10	84	770	2.7	0.59 (0.47)	0.26	0.33	0.56

[a] Product 1 was isolated after a reaction time of 96 h whereas products 2−4 were obtained after 139 h. [b] Initial water content measured for each hydrolysis reaction was 0.13 mmol. [c] Conversion of ϵ-CL was determined as the product weight after vacuum oven drying relative to the initial weight of ϵ-CL charged. [d] Mole fraction of carboxylic acid and butyl amide chain ends. [e] Total number of chains was calculated from the equation $N_p = (v \times \rho \times C_m \times 1000)/M_n$, where v and ρ are the volume and density of ϵ-CL, respectively, and C_m is the fractional monomer conversion.

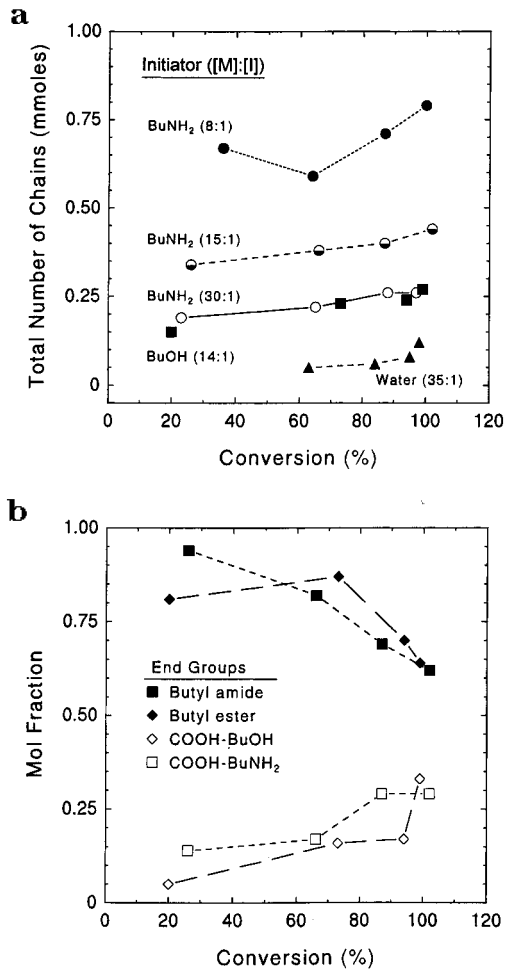
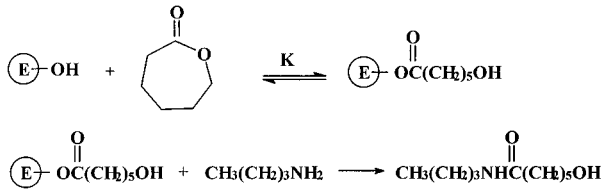


Figure 6. PPL-catalyzed ϵ-CL polymerizations carried out to variable percent conversions. Effects of the nucleophile type and concentration on (a) total number of chains and (b) mole fraction of chain end functional groups.

the general trend observed was an increase in total number of polymer chains, N_p, with conversion. For reactions with butanol and butylamine, this increase in chain number corresponded with a decrease in the mole fraction of butyl ester and butyl amide end groups and an increase in carboxylic acid chain ends (see Figure 6b). In addition, since the mole fraction of carboxylic acid end groups increases with conversion, we believe that the observed increase in N_p during the polymerization results from reactions with water. Interestingly, based on polymer yield and end group structure (Figure 6), it was found that only 37 and 52% of the butanol was consumed after 4 and 96 h, respectively (i.e., 20 and 100% ϵ-CL conversion). In contrast, butylamine (ϵ-CL/

Scheme 1



$BuNH_2$ ratio 15/1) was completely consumed within 4 h or at 26% monomer conversion. For example, the N_p at this conversion was equivalent to the amount of butylamine added (0.30 mmol) and the mole fraction of butyl amide chain ends was 0.94. Thus, butylamine was rapidly consumed (reaction times < 4 h) largely via enzyme catalysis and the rate of initiation was faster than the rate of propagation. This is supported by studies described above carried out to low conversion where *N*-butyl-6-hydroxyhexanamide was formed without noticeable chain growth. The mechanism for enzyme-catalyzed chain initiation reactions between butylamine and ϵ-CL conceivably proceeds via an enzyme-activated mechanism[3,6] as shown in Scheme 1.

Furthermore, the cumulative results of our work with PPL-catalyzed ϵ-CL polymerization indicates that this is a chain polymerization with slow propagation.[3]

Chain Degradation by Enzyme-Catalyzed Hydrolysis. Experiments were performed to determine whether the observed increase in polymer chains noted above results from the hydrolysis of PCL during the course of the reaction. PCL was synthesized as described above (see Experimental Section) in heptane using PPL as catalyst (ϵ-CL/$BuNH_2$ of 15/1). The results of this work are summarized in Table 1. The M_n values for reactions conducted for 96 and 139 h (products 1 and 2, respectively) without added water were almost identical (1150 and 1130 g/mol, respectively). Thus, the degradation of chains at reactions times between 96 and 139 h without water addition was not significant. This is not surprising since the initial water contents of reactions (0.13 mmol) as measured by Karl Fischer titrations (see Experimental Section) were almost exhausted by 96 h. However, when 0.56 and 1.10 mmol of water were added at 96 h, the polymers that resulted after an additional 43 h incubation period (products 3 and 4, respectively) showed substantial decreases in M_n (to 840 and 770 g/mol, respectively), increases in total chains (to 0.54 and 0.56, respectively), and increases in the fraction of carboxylic acid end groups (to 0.57 and 0.59, respectively) relative to product 2. The increase in total chains upon addition of water (products 3 and 4 relative to product 2) corresponds well with the increase in reacted water, which indicates that reactions

between water and polymer do not occur specifically at chain ends but, instead, take place by intrachain cleavage. The decrease in M_w/M_n values of products 3 and 4 relative to products 1 and 2 further supports that intrachain cleavage reactions occur and take place by events of random chain scission. Since PCL does not show significant loss in molecular weight in the absence of enzyme under the incubation conditions used herein,[12] the observed hydrolysis of PCL chains occurs by enzyme catalysis. Therefore, it is reasonable to conclude that enzyme-catalyzed hydrolysis of PCL chains occurs during the course of PPL-catalyzed ϵ-CL polymerizations and may, in part, be responsible for product molecular weights that either decrease or remain unchanged at high monomer conversion (see Figure 5).

Living/Immortal Characteristics and Derivation of a Rate Expression. Living polymerizations are normally defined as reactions (propagating centers) that do not terminate or undergo chain transfer. In the absence of termination, propagation can be defined by a first-order rate law, which upon rearrangement and integration yields a linear expression. The linearity of M_n as a function of conversion may reflect the lack of chain transfer. Therefore, the living/immortal nature of PPL-catalyzed ring-opening polymerizations carried out using butylamine (0.30 mmol, ϵ-CL/$BuNH_2$ ratio 15/1) in heptane was assessed by constructing plots of $\log\{[M]_0/[M]_t\}$ versus time and M_n versus monomer conversion (see Figure 7). For the above, $[M]_0$ is the initial concentration of monomer and $[M]_t$ is the monomer concentration after time t. Since it was demonstrated herein that a considerable amount of monomer was consumed during chain initiation (termed $[M]_i$), the values of $\log\{[M]_0/[M]_t\}$ were corrected and plotted as $\log\{([M]_0 - [M]_i)([M]_t - [M]_i)^{-1}\}$ so that monomer consumption during propagation was analyzed. The plot of $\log\{([M]_0 - [M]_i)([M]_t - [M]_i)^{-1}\}$ versus time (Figure 7a) was linear ($r^2 = 0.997$), indicating (i) no termination and (ii) that monomer consumption follows a first-order rate law.

Plots of M_n versus monomer conversion (see Figure 7b) were constructed using experimental and calculated M_n values. In this case, calculated M_n values were obtained using eqs 1a and 1b:

$$M_n = [M]_0/[I] \times C_m \times m_{CL} \quad (1a)$$

$$M_n = [M]_0/[I'] \times C_m \times m_{CL} \quad (1b)$$

where [I] is the concentration of butylamine charged, [I′] is the total concentration of butylamine and water that reacted (determined by ^1H NMR), C_m is the fractional monomer conversion, and m_{CL} is the molar mass of ϵ-CL. The line generated by using eq 1a to calculate M_n deviated significantly from the experimental data. However, there was excellent agreement between the experimental data and the line generated using eq 1b to calculate theoretical M_n values. Knowing that the initiator concentration is best described by [I′], these results suggest that chain transfer did not occur.

It has been demonstrated[13,14] that for low M_n values, one may not observe the effects of chain transfer in plots of M_n versus conversion. This is particularly true if chain transfer is slow relative to propagation ($k_{tr} \ll k_p$), with the latter value masking the former. A direct experimental approach to investigate this possibility would be to analyze the results from higher molecular weight products. Since this was not possible during the course of this study, at present we can at least conclude

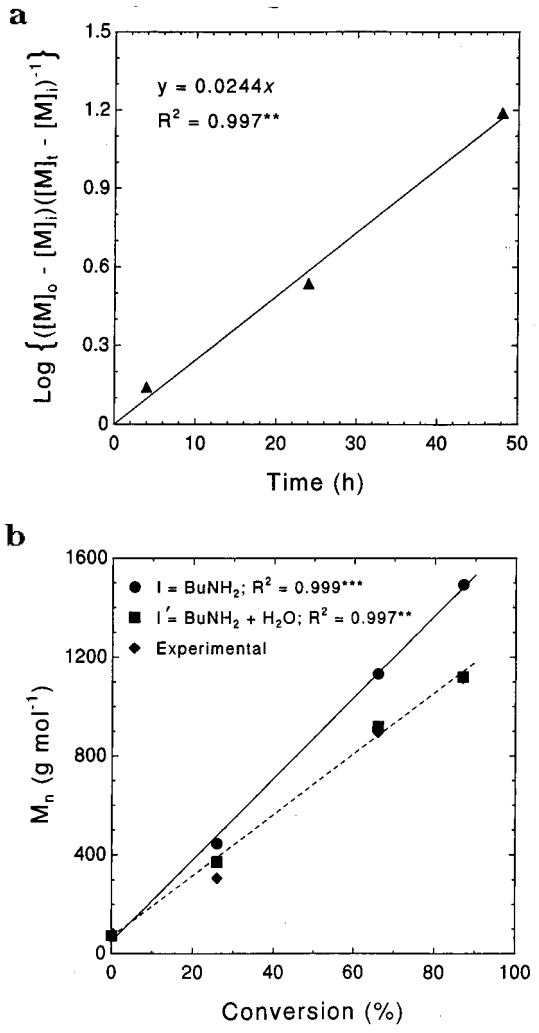
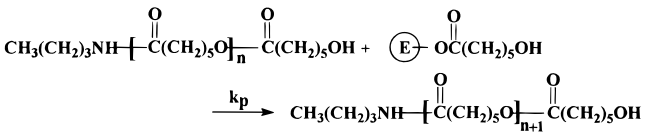


Figure 7. Plots of (a) $\log\{([M]_0 - [M]_i)([M]_t - [M]_i)^{-1}\}$ versus time and (b) M_n versus percent conversion for PPL-catalyzed ϵ-CL polymerizations using butylamine (ϵ-CL/$BuNH_2$ = 15/1).

that this system provides "controlled" polymerizations. We have chosen to define the following rate expressions based on living characteristics (homogeneous equations).

From this investigation, it is known that the rate at which monomer is consumed shows almost no dependence on the type of nucleophile employed nor its concentration (see Figure 4). For example, varying the ϵ-CL to butylamine ratio so that the molar ratios were 8/1, 15/1, and 30/1 results in similar curves for percent conversion as a function of time (Figure 4b). This implies that the propagation rate, R_p, is independent of [I]. Furthermore, R_p does not change as N_p varies to a large extent as a result of the type and concentration of nucleophile used (see Figures 4 and 6a). Therefore, the concentration of chains ends, [R∼OH], appears to be zero order with respect to the rate. One possible explanation for this observation is that, for all of these polymerizations, the concentration of propagating chain ends at the enzyme−substrate interface is at saturation with respect to enzyme-activated monomer. Based on our results for polymerizations carried out with and without PPL, it was concluded that the rate of monomer consumption was a function of the catalyst concentration.[15] From these results, an experimental rate expression was defined as $R_p = k_{obs}[Cat]^x[M]^1[R{\sim}OH]^0$.

Scheme 2



The first-order rate law as indicated by the linear relationship shown in Figure 7a supports the mechanism for propagation shown above and discussed previously in ref 3 (Scheme 2).

To give a theoretical rate equation from the propagation mechanism in Scheme 2, $R_p = k_p[AM][R{\sim}OH]$, where [AM] is the concentration of the acyl-enzyme intermediate formed in the initiation step. Combining the schemes for chain initiation and propagation, [AM] can be determined from the equilibrium in the first step of chain initiation (Scheme 1) and is equal to K[Cat][M]. Substituting into the rate equation knowing that [R∼OH] is zero order and monomer concentration is first order leads to the rate law $R_p = k_pK[Cat]^x[M]^1[R{\sim}OH]^0$. Rearranging this equation, integrating it with respect to time, and plotting $\log\{[M]_0/[M]_t\}$ versus time should yield a linear relationship with a slope equal to $k_pK[Cat]^x$. Since the slope of the log plot was indeed constant (see Figure 7a), this indicates that the catalyst concentration is invariable during the course of the polymerization. This is reasonable since a catalyst, by definition, is not consumed. Furthermore, we have shown in a previous study that the activity of PPL appeared unchanged when it was recovered and used again for a second ϵ-CL polymerization.[16] Thus, the experimentally derived rate expression is consistent with that derived from the proposed mechanism for enzyme-catalyzed ϵ-CL polymerization.

Enzyme-catalyzed ϵ-CL polymerization was also compared to the immortal polymerizations described by Inoue.[9] Using porphinato catalysts for the ring-opening of lactones, Inoue defined the requirements for immortal polymerization as follows: (i) no termination; (ii) presence of exchange reactions such that X_n equals $[M]_0/[I']$, where [I′] is the sum of multiple nucleophiles; (iii) N_p exceeds that of the initiator concentration and equals the total nucleophile concentration; and (iv) rapid exchangeability, where the rate of initiation exceeds that of propagation and polymer with a narrow molecular weight distribution results. Interestingly, it was shown herein that when the initiator concentration was taken as the total of the nucleophiles (butylamine and water), $M_n = [M]_0/[I'] \times C_m \times m_{CL}$. This implies that N_p equals the sum of the concentrations of butylamine and reacted water. Moreover, if the initiator concentration [I] is assumed to be equal to the concentration of butylamine, the predicted total N_p (i.e., 0.30 mmol at 100% conversion) is less than that actually observed (Figure 6a). The M_w/M_n values measured for these reactions were in the range of 1.5−5.0, which is considerably greater than that reported by Inoue (∼1.13). We believe that broadened molecular weight distributions observed in this work result in part from slow initiation by the nucleophiles water and butanol as well as by enzyme-catalyzed chain hydrolysis. Furthermore, in regards to exchangeability, we believe that the rate at which propagating PCL chains continue to grow is controlled at the active site through a process of rapid exchangeability of chain ends. This is supported by the fact that accumulation of the monoadduct, *N*-butyl-6-hydroxyhexanamide, was not observed by GPC. Therefore, our data indicate that enzyme-catalyzed polymerizations of ϵ-CL meet many of the requirements introduced by Inoue[9] for immortal polymerizations.

Work is currently in progress to determine (i) [AM], (ii) the reaction order with respect to the rate law, and (iii) the kinetics of the ring-opening of ω-pentadecalactone using the lipase from *Pseudomonas sp.* (PS-30) which resulted in polyesters with an M_n of 50 000 g/mol.[17]

Summary of Results

Studies using different nucleophiles showed that the rate of initiation by butylamine is much faster than by butanol or water. Investigations to low monomer conversion showed that butylamine is rapidly consumed to form *N*-butyl-6-hydroxyhexanamide prior to chain growth. This chain initiation step occurred in large part by enzyme catalysis. The hydrolysis of chains was studied by the addition of water to reactions at 96 h. Based on this work and the observation that product molecular weights decrease at high monomer conversion, it was concluded that PPL-catalyzed hydrolysis of PCL chains occurs during the course of polymerizations. The living/immortal nature of PPL-catalyzed ϵ-CL ring-opening polymerizations (ϵ-CL/$BuNH_2$ ratio 15/1) in heptane was studied by constructing plots of $\log\{[M]_0/[M]_t\}$ versus time and M_n versus monomer conversion. The linearity of these plots indicated (i) a lack of termination, (ii) monomer consumption followed a first-order rate law, and (iii) the absence of chain transfer. Given the ambiguity of chain transfer, we limit our description of the system as providing "controlled" polymerizations. The rate of monomer conversion showed almost no dependence on the type or concentration of nucleophile. Thus, it was concluded that R_p is independent of [I]. Furthermore, R_p was found to be independent of the total number of chains. Therefore, the concentration of chains ends was zero order with respect to the rate. From this kinetic data, an expression for R_p was derived which is consistent with that from the proposed enzyme-catalyzed polymerization mechanism. Further analysis showed that N_p equaled the sum of the concentrations of butylamine and reacted water. Broadened molecular weight distributions observed in this work but not in that of Inoue are believed to be due in part to slow initiation by the nucleophiles water and butanol as well as by enzyme-catalyzed chain hydrolysis. The above kinetic analyses in conjunction with the relationship between molecular weight and the total concentration of multiple initiators suggests that ϵ-CL polymerization by PPL catalysis shares many features with that of immortal polymerizations.

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(15) The rate dependence on the concentration of the catalyst was also observed in experiments where the concentration of PPL was decreased from 750 to 375 mg for ϵ-CL polymerization initiated with water (0.12 mmol). The results for percent monomer conversion decreased from 100 to 86% after a 96 h reaction time (unpublished results).
(16) See citation 16 in ref 3.
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