Enzyme-Catalyzed ϵ-Caprolactone Ring-Opening Polymerization

Renée T. MacDonald, Satish K. Pulapura, Yuri Y. Svirkin, and Richard A. Gross*

Department of Chemistry, University of Massachusetts Lowell, One University Avenue, Lowell, Massachusetts 01854

David L. Kaplan and Joseph Akkara

U.S. Army Natick RD&E Center, Biotechnology Division, STRNC-YM, Kansas Street, Natick, Massachusetts 01760-5020

Graham Swift and Steven Wolk

Rohm & Haas Company, Morristown Road, Spring House, Pennsylvania 19477

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ABSTRACT: The polymerization of ϵ-caprolactone, (ϵ-CL) using porcine pancreatic lipase (PPL) as the catalyst was studied. Polymerization reactions (4 days, 65 °C) of ϵ-CL at ~10% (w/v) concentrations in dioxane, toluene, and heptane using butanol as an initiating species (monomer/butanol ratio = 14.7) gave poly(ϵ-caprolactone) (PCL) with M_n values (by GPC) of 313, 753, and 1600, respectively. Monomer conversion to PCL for these polymerizations was 33, 55, and 100%, respectively. M_n measurements of PCL products by ^{1}H NMR end group analyses were slightly lower (by a factor of ~0.9) than the values obtained by GPC. Polymerizations conducted in heptane at 37, 45, 55, and 65 °C showed the highest extent of monomer conversion at 65 °C. Therefore, subsequent studies were conducted at 65 °C in heptane. For a polymerization carried out with a 15/1 monomer/butanol ratio and ~0.29 mmol of water, ~70 and ~100% of the monomer had been converted to PCL by reaction times of 24 and 96 h, respectively. Polymer molecular weight increased slowly with conversion, suggesting that this is a chain polymerization with rapid initiation and slow propagation. Increases in the ϵ-CL/butanol ratio from 15/1 up to where no butanol was added showed only a modest increase in product molecular weight from 1600 to 2700. This was explained by the fact that the water present in polymerizations was active in chain initiation. Variation in the monomer/butanol ratio at constant water concentration resulted in PCL chains with 0−0.65 mol fraction of butyl ester and 0.33−0.86 mol fraction of carboxylic acid chain end groups (by ^{1}H NMR analyses). The presence of water concentrations in polymerization reactions above that which is strongly enzyme bound is believed to be an important factor which limited the formation of PCL chains of significantly higher molecular weight.

Introduction

Enzyme-catalyzed ring-opening and closure of lactones have been reported. Gutman and Bravdo[1] carried out the enantioconvergent lactonization of symmetrical hydroxy diesters which exploits the prochiral stereospecificity of lipases. In their work, they made use of the lipase from *Pseudomonas fluorescens* (PF) as well as porcine pancreatic lipase (PPL) in hexane to obtain lactones in up to 98% enantiomeric excess. By using commercial enzyme preparations derived from different biological systems, either of the lactone antipodes were prepared with a substantial enantiomeric excess. Gutman and co-workers[2] used PPL in hexane, ether, and toluene to catalyze the lactonization of a number of γ-hydroxy acid esters, achieving enantiomeric excess values >94%. Blanco et al.[3] have reported 60−90% enantiomeric excess values for the enzymatic resolution of substituted racemic γ-, δ-, and ϵ-lactones. In their work, they used PPL, horse liver esterase, and pig liver esterase.

Investigations directed toward enzyme-catalyzed polyester synthesis have been reported by a few laboratories. Gutman and co-workers[4] reported that oligomerization occurred as a competing reaction while carrying out an enzyme-catalyzed lactonization of an ω-hydroxy ester. Work by Kilbanov and co-workers[5] was directed toward stereoselective oligomerization reactions catalyzed by lipases in organic media. Morrow and co-workers[6] synthesized optically active polyesters (M_w = 7900, >95% optical purity) from 1,4-butanediol and a racemic epoxide-substituted diester using PPL in anhydrous diethyl ether. Dordick and co-workers prepared sucrose polyesters (M_w = 2100) and poly(ester amides).[7] In addition, Kobayashi et al. synthesized cellulose by the polymerization of β-D-cellobiosyl fluoride using a cellulase enzyme catalyst.[8]

An interesting route to enzyme-catalyzed polymer-forming reactions is that of ring-opening chemistry. Inherent advantages of this approach are that upon monomer ring-opening a leaving group is not produced that would lead to reactions of polymer degradation, the ring strain of the system could be altered to modulate polymerization kinetics, and chiral lactones could be polymerized by enantioselective mechanisms. Two communications have recently been published which support the utility of this route. Specifically, Uyama and Kobayashi have reported the polymerization of δ-valerolactone and ϵ-caprolactone (ϵ-CL) using the lipase enzymes from *P. fluorescens*, *Candida cylindracea*, and PPL.[9] The polymerizations were all carried out in bulk for 10 days. Of the three lipases studied, that from *P. fluorescens* gave the highest percent monomer conversion (92%) and molecular weight (M_n = 7700) for ϵ-CL polymerization. Study of the poly(ϵ-caprolactone) (PCL) structure for one of the *P. fluorescens*-catalyzed preparations showed that the end groups were hydroxyl and carboxylic acid, respectively. Also, Knani et al.[10] studied ϵ-CL ring-opening polymerization catalyzed by

* To whom correpondence should be addressed.

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Table 1. Products Obtained by Enzyme-Catalyzed ϵ-CL Polymerization at 65 °C after a 4 Day Reaction

product	solvent[f]	ϵ-CL[a] (nmol)	water content[a] (mmol)	added butanol (mmol)	M_n (GPC)[b]	M_w/M_n (GPC)	M_n (^{1}H NMR)[c]	mol frac of acid end groups[c]	mol fract of butyl ester end groups[c]	reacted water[d] (mmol)	% conv
1	dioxane	4.40	0.29	0.30	nd[h]	nd	310	nd	nd	nd	nd
2	toluene	4.40	0.29	0.30	nd	nd	750	nd	nd	nd	nd
3	heptane	4.40	0.29	0.30	1600	1.8	1380	0.33	0.65	0.12	100
4	heptane	4.40	0.29	0.065	1900	1.9	1700	0.66	0.16	0.20	100
5	heptane	4.40	0.29	0.032	2300	1.9	1590	0.73	0.13	0.23	100
6	heptane	4.40	0.29	0	2700	1.9	2710	0.86	0	0.16	100
7	heptane	4.40	0.64	0.30	710	1.5	608	0.53	0.33	0.38	nd
8[g]	not used	8.80	0.64	0	1100	1.4	1030	0.87	0	0.44	nd
control[e]	heptane	4.40		0.30							

[a] Analyzed by Karl Fischer titration (see Experimental Section). Standard deviation based on triplicate analyses was ≤0.05 mmol. [b] Determined by GPC (see Experimental Section). [c] By ^{1}H NMR spectral integration (see Experimental Section). [d] Reacted water was determined by multiplying the number of polymer chains (in mmol) by the mole fraction of acid end groups. [e] Control was performed using PPL that had been denatured by heating a PPL aqueous solution at 100 °C for 5 min (see ref 14 for details). [f] Solvent volume = 5 mL (10% w/v monomer concentration). [g] Product preparation carried out without solvent addition (in bulk). [h] nd = not determined.

PPL and initiated by methanol. These workers carried out their studies at 40 °C and reported time periods up to 26 days for complete conversion of monomer to the products dilactone and PCL.

The present work explores important experimental variables and resulting effects on polymer structure for ϵ-CL polymerization to form PCL using PPL as catalyst. Investigations of solvent and temperature effects on the percent monomer conversion to PCL are reported. The time dependence of ϵ-CL conversion and product molecular weight is investigated. Also, effects of the reaction water content and the monomer/butanol initiator ratio on the product molecular weight, chain end group structure, and reacted water are studied. The results obtained are used to discuss mechanistic features of the polymerization.

Experimental Section

Instrumental Methods. Nuclear Magnetic Resonance (NMR). Unless otherwise specified, proton (^{1}H) NMR spectra were recorded on a Bruker AMX500 spectrometer. Each sample for NMR analysis was prepared by dissolving 5 mg of polymer in 0.8 mL of $CDCl_3$. All data were acquired at room temperature using a standard one-pulse acquisition. Each spectrum was acquired using an excitation pulse of 3 μs (≈35°), a sweep width of 6024 Hz, a relaxation delay of 6.0 s, 64 scans, and 32K complex data points. Spectra were referenced to the residual $CHCl_3$ peak (7.24 ppm) and integrated using the subroutine provided with the Bruker UXNMR software.

^{1}H NMR spectra for the determination of percent monomer conversion as a function of polymerization time (Figure 3) were recorded on a Bruker AC spectrometer at 250 MHz. NMR chemical shifts in parts per million (ppm) are reported downfield from 0.00 ppm using tetramethylsilane (TMS) as an internal reference. Polymer spectral acquisitions were conducted as 5.0% w/v $CDCl_3$ solutions at room temperature using the following parameters: 298 K, 32K data points, 2 s relaxation delay, line broadening 0.488 Hz, 50-100 transients.

Molecular Weight, End Group Analyses, and Monomer Conversion by ^{1}H NMR Spectroscopy. The degree of polymerization (DP) and the corresponding M_n values of the synthesized polymers were measured by comparison of the intensities (using spectrometer integration values) of the methylene protons adjacent to the hydroxyl end group (protons a; see Figure 1) at 3.65 ppm with respect to the methylene protons adjacent to the oxygen of intrachain repeat units (protons b; see Figure 1) at 4.05 ppm. The mole fraction of acid and butyl ester end groups was measured by first converting the product chain end free acids to the corresponding methyl ester functionalities using diazomethane. The preparation of diazomethane and its subsequent use for product derivatization was performed following a literature procedure.[11] The spectrometer integration values of the chain end methyl groups of methyl and butyl esters (protons l and k, respectively; see Figure 1b) at 3.65 and 0.9 ppm, respectively, were normalized to two protons and then compared to the intensity of protons a (see above). The relative intensities of ^{1}H NMR signals corresponding to protons a and l was determined from spectral expansions of the 3.6–3.7 ppm region (see Figure 1b) by carrying out peak shape approximations using Gaussian curve fitting and then by cutting and weighing peaks.

Conversion of ϵ-CL to PCL was determined by comparison of the spectral integration intensities of the triplet at 2.7 ppm corresponding to the methylene group adjacent to the carbonyl of ϵ-CL with respect to the PCL intrachain protons b at 4.05 ppm.

Molecular Weight Measurements by Gel Permeation Chromatography (GPC). The number- and weight-average molecular weights (M_n and M_w, respectively) were determined by gel permeation chromatography (GPC). Studies by GPC were carried out 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 placed in series. Chloroform was used as the eluent at a flow rate of 1.0 mL/min. Sample concentrations of 0.4% (w/v) and injection volumes of 100 μL were used. Polystyrene standards (molecular weights of 300, 1000, 2500, 4000, 8000, 24 000, 90 000, 207 000, 507 000, and 1 450 000 (Polysciences)) with low dispersities were used to generate a calibration curve. When a GPC peak having an identical retention time to that of ϵ-CL was observed in chromatographs of product samples, the peak was not included as part of the product peak area for molecular weight calculations.

Water Analyses. Water contents were measured using a Mettler DL18 Karl Fischer titrator with Hydranal-Titrant 5 (methanol/I_2) and Hydranal-Solvent (methanol/imidazole/sulfur dioxide). The reaction components—ϵ-CL, butanol, heptane, toluene, and dioxane—were each measured by injecting approximately 0.5 mL into the Hydranal-Solvent system. For PPL, an estimate of the water content was obtained by stirring 750 mg of the enzyme in 10 mL of either methanol (Aldrich, 99+%, anhydrous grade) or dimethyl sulfoxide (Aldrich, 99+%, anhydrous grade) for 16 h, separating the enzyme by filtration, and determining the difference in the water contents of the filtrate and solvent controls (controls did not contain enzyme but were treated in the same manner as the solvents containing the enzyme). Enzyme water contents determined using either dimethyl sulfoxide or methanol as above were almost identical.

The water contents shown in Table 1 (in mmoles) are the sum of water values measured for each component in the polymerization reactions. The additive water content of all components in the reactions other than PPL was only ~5% of that for PPL.

Synthetic Procedures. ϵ-Polymerizations Using Dried Reagents. Polymerizations were carried out in the presence of heptane using variable butanol concentrations, with known quantities of water and for different time periods (see Table 1 and Figure 3 below). Also, reactions were carried out in dry

toluene (distilled from Na°) and dioxane (distilled from Na°/benzophenone). A representative procedure where dried reagents were used is as follows. PPL (Sigma Chemical Co., Type II Crude, manufacturer reported activity = 128 units/mg of protein ≈ 25% protein, measured using the olive oil method) was sieved using mesh screens to obtain a particle size of between 80 and 100 mesh. The sieved enzyme (750 mg) was transferred into an oven-dried 50 mL Erlenmeyer flask (the reaction flask) and dried (0.1 mmHg, 16 h, 25 °C) in a vacuum desiccator. The reaction flask with dried enzyme was then immediately septum stoppered and purged with argon. To the reaction flask were added via syringe dry heptane (5 mL, stirred over anhydrous $MgSO_4$, filtered, and then fractionally distilled over Na° under argon), dry butanol (0.03 mL, 0.30 mmol, stirred over anhydrous $MgSO_4$, filtered, and then fractionally distilled over Na° under argon), and ϵ-CL (0.5 mL, 4.40 mmol, Aldrich Chemical Co., fractionally distilled over CaH_2 [83 °C, 1.7 mmHg]). The septum-stoppered (wrapped with Teflon and secured in place using tightly wound stainless steel wire) reaction flasks were placed into a shaker incubator (65 °C, 200 rpm) for reaction times from 4 to 96 h. The reactions were terminated by separation of the enzyme catalyst from product and/or residual reagents by vacuum filtration. The filtered enzyme was then washed with several portions of chloroform, and the filtrates were combined. Volatile solvents and butanol were removed from the product polymer and residual monomer by rotary evaporation (50 °C, 150 mmHg, 1 h). Further removal of solvents, butanol, and ϵ-CL was then carried out using a diffusion pump and drying pistol apparatus (16 h, 55 °C, 5 μmHg). The resulting products were then studied by ^1H NMR and GPC (see above for methods used). Analysis of monomer conversion as a function of polymerization time (Figure 3a) was carried out on samples after rotary evaporation.

ϵ-CL Polymerizations with Reagents That Were not Dried. Products **7** and **8** in Table 1 were obtained using the following method. Heptane and butanol were purified by fractional distillation without the use of drying agents. ϵ-CL (Aldrich, 99% purity) and PPL (see above) were used as received. Transfers of all reagents were carried out under normal atmospheric conditions. This, of course, resulted in polymerization reactions which contained relatively higher water concentrations (see Table 1). The reaction conditions and method for termination and isolation of products **7** and **8** were identical to those described above for polymerizations carried out using dried reagents.

Results and Discussion

^1H NMR Structural Analysis and GPC Measurements of Products Formed. The ^1H NMR spectra of product **3** (see Table 1) before and after diazomethane chain end derivatization are shown in parts a and b of Figure 1, respectively. The formation of methyl ester chain end groups (protons l) by derivatization of product carboxylic acid functionalities with diazomethane gave a new ^1H NMR signal at ~3.65 ppm (see expanded spectral region, Figure 1b). ^1H NMR signals for protons b–f (see Figure 1b) are consistent with those previously reported by others for PCL repeat units.[12] Also, assignment of the ^1H NMR hydroxyl end group signals ($HOCH_2$, protons a) at ~3.65 ppm is based on the work of others.[12] The disappearance after diazomethane derivatization of a complex multiplet centered at approximately 2.35 ppm (see Figure 1a,b) suggests that the 2.35 ppm signals can be assigned to protons g (see Figure 1a). Assignment of signals due to butyl ester end group protons was made using known chemical shifts of model compounds.[13] Specific details of the methods used for quantitation of the percent conversion, the mole fraction of chains with acid butyl ester end groups, and the M_n of products obtained herein are given in the Experimental Section.

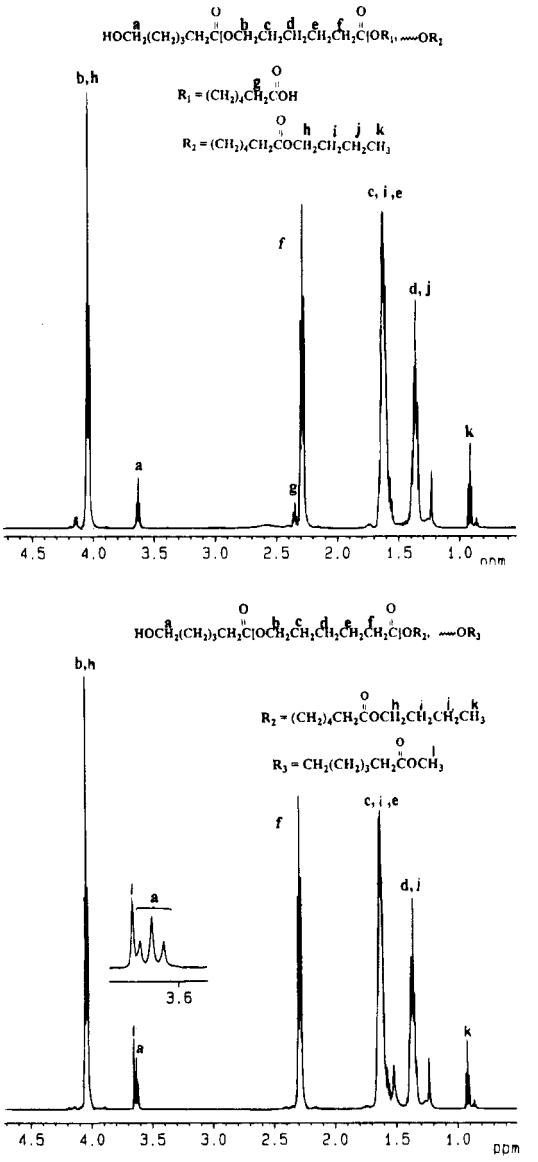


Figure 1. ^1H NMR spectra (500 MHz) in $CDCl_3$ of product **3**: (a, top) as recovered; (b, bottom) after diazomethane chain end derivatization.

Previous studies by Knani et al. showed that ϵ-CL (7.7% w/v in hexane) was converted by PPL enzyme catalysis (40 °C, 624 h reaction time) to dilactone and PCL in relative amounts (by weight) of ~9 and ~91%, respectively.[10] Knani et al. determined the relative amounts of PCL and dilactone by ^1H NMR using triplets at 4.14 and 2.35 ppm corresponding to the dilactone methylene groups adjacent to the ester oxygen and carbonyl, respectively. In Figure 1a, signals with greater complexity than that of first-order triplets were observed at 4.14 and 2.35. However, after derivatization of product **3**, the signals at 4.14 and 2.35 ppm almost disappeared (see Figure 1b). Similarly, disappearance of signals at 4.14 and 2.35 ppm after diazomethane derivatization was observed for other products reported herein. Therefore, utilizing the ^1H NMR assignments reported by Knani et al.[10] for identification of dilactone, we were not able to confirm the transformation of ϵ-CL to dilactone for the polymerization reaction conditions given in Table 1.

On average, M_n measurements of PCL products by ^1H NMR end group analysis (see Experimental Section for method used) were slightly lower (by a factor of ~0.9) than the values obtained by GPC (see Table 1). GPC

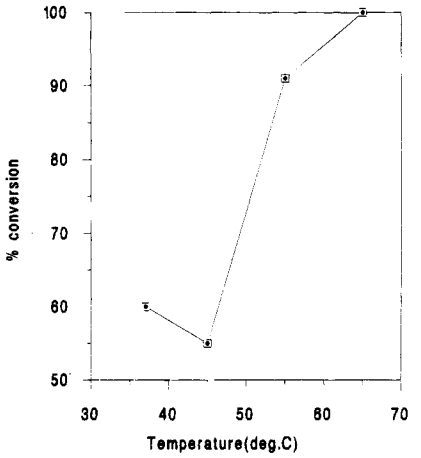


Figure 2. Percent ϵ-CL conversion as a function of temperature for a 96 h polymerization using reaction conditions as given for product **7** in Table 1 without butanol addition.

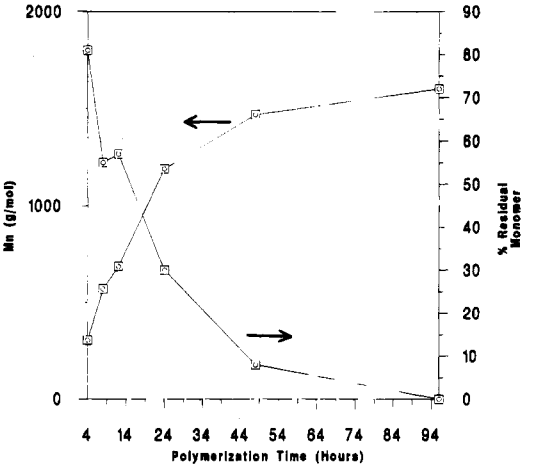


Figure 3. Percent ϵ-CL conversion and product M_n (by ^{1}H NMR) as a function of reaction time for the polymerization reaction to form product **3**.

M_n measurements showed small values of standard deviation (see Table 1) and, therefore, are considered reliable to determine effects on product molecular weight resulting from systematic changes in the polymerization parameters used. For studies below on the time dependence of monomer conversion and product M_n, only ^{1}H NMR results of M_n were obtained and presented. In most cases (with the exception of product **5**, see Table 1) when both GPC and ^{1}H NMR M_n values were measured, ^{1}H NMR end group M_n values support the trends observed by GPC.

Effects of Solvent and Temperature. The effect of solvent character on the PPL-catalyzed polymerization of ϵ-CL was studied. The reaction conditions used were identical to those described for the synthesis of product **3** shown in Table 1 except that dioxane and toluene were investigated in place of heptane. The percent conversions determined by ^{1}H NMR for the reactions in dioxane, toluene, and heptane after 4 day reaction times were 33, 55, and 100%, respectively. The product M_n values by ^{1}H NMR analysis for these polymerization reactions were 310, 750, and 1380, respectively (see Table 1). When solvent was omitted from the polymerization reactions (carried out in bulk), PCL with an M_n of 1100 (by GPC) was obtained (see product **8**, Table 1). From these studies, it was thus determined that the use of heptane or the absence of solvent was preferred in the reaction. It is important to note that reactions conducted in the presence of heptane are rather complex since the solubility of ϵ-CL in heptane is low ($\approx$3 g of ϵ-CL dissolves in 100 g of heptane at 65 °C).

The effect of reaction temperature on PPL-catalyzed ϵ-CL ring opening was investigated. The reaction conditions used for this work were identical to those described for product **7** (see Table 1 and the Experimental Section) with the exception that butanol was not added. The extent of monomer conversion was measured by ^{1}H NMR spectral integration (see Experimental Section). Figure 2 shows that the percent conversion increased from ~60 to 100% as the reaction temperature was increased from 37 to 65 °C. This agrees with previous work by Uyama and Kobayashi, who, using a lipase from *P. fluorescens* to catalyze ϵ-CL polymerization, found increased percent conversion and product molecular weight at 75 °C relative to 30, 45, and 60 °C polymerization temperatures.[9] To determine that the increase in ring-opening reactions at 65 °C was indeed due to enzyme-catalyzed processes as opposed to non-enzyme-mediated reactions, a control was performed (see Table 1) where denatured PPL was used in place of catalytically active PPL.[14] Under these conditions, ring opening of ϵ-CL was not detected by ^{1}H NMR spectroscopy. Based on these results, it was concluded that ϵ-CL polymerization observed at 65 °C reaction temperatures was due to enzyme catalysis. Furthermore, the addition of heptane as opposed to toluene and dioxane gave higher monomer conversion and product molecular weight. Thus, subsequent studies described below were carried out at 65 °C with the addition of heptane.

Effects of Reaction Time, Initiator, and Water Concentration. Polymerizations were carried out for reaction times from 4 to 96 h using conditions identical to those for the formation of product **3** (see Table 1). The time dependences of ϵ-CL conversion and PCL M_n are shown in Figure 3. From Figure 3 it was observed that by 24 and 96 h reaction times, ~70 and ~100%, respectively, of the ϵ-CL had been converted to PCL. Interestingly, under polymerization conditions similar to those used in this work but at a 40 °C polymerization temperature, a 624 h reaction time was required to reach ~100% monomer conversion.[10] Figure 3 also shows that the polymer molecular weight increases slowly with conversion, suggesting that this may be a chain polymerization with rapid initiation and slow propagation. Furthermore, since only a small product molecular weight increase (1500 to 1600) was observed between 90 and 100% conversion (see Figure 3), it appears unlikely that condensation reactions play an important role in chain growth.[15] Therefore, assuming PPL enzyme activity was maintained to 96 h under the reaction conditions employed,[16] it can be concluded that the predominant reaction leading to productive chain growth for PPL-catalyzed ϵ-CL polymerization is ϵ-CL ring opening.

If it is assumed that butanol added to the polymerization reaction is the only initiator species, then products **3**, **4**, **5**, and **6** were formed under conditions where the initial monomer to initiator ratio ($[M]_0/[I]_0$) was 15/1, 67/1, 138/1, and ∞ ($[I] = 0$), respectively (see Table 1). Equation 1, which applies in the absence of chain transfer and termination, was then explored to analyze the dependence of DP on conversion and initiator concentration. This approach appears reasonable with the mechanism proposed below. As would be expected,

a general trend of increased M_n (1600, 1900, 2300, and 2700, respectively) was observed. However, the magnitude of change in M_n for large increases in $[M]_0/[I]_0$ values was smaller than that expected using eq 1:

$$\text{degree of polymerization (DP)} = ([M]_0 - [M]_t)/[I]_0 \quad (1)$$

where $[M]_t$ is the monomer concentration at time t = 96 h ($[M]_t = 0$ for reactions leading to products **3**–**6**). An explanation for the small dependence of M_n on $[M]_0/[I]_0$ is the existence of an active initiator species other than butanol. Structural evidence that both water and butanol initiate ϵ-CL ring-opening polymerization is the formation of polymer chains with variable mole fractions of carboxylic acid and butyl ester chain ends (see Table 1, Figure 1, and discussion below). Studies by Uyama and Kobayashi using *P. fluorescens* to catalyze ϵ-CL polymerization in the absence of an added alcohol initiator also showed the formation of chains with hydroxyl and carboxylic acid functionalities at the respective chain ends.[9] Thus, the DP is more accurately described by the following relationship:

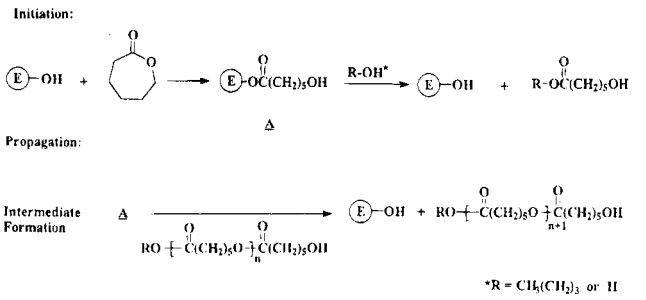
$$\text{DP} = \frac{[M]_0 - [M]_t}{[\text{butanol}] + [\text{reacted water}]} \quad (2)$$

The higher M_n for product **3** relative to product **7** (1600 and 710, respectively) for reactions carried out with 0.29 and 0.64 mmol of water, respectively, is consistent with chain initiation by water. The application of eq 2 is more difficult than might be expected since a critical concentration of water required for enzyme activity is tightly bound[17,18] where additional water, above this critical concentration, will have variable degrees of affinity for the enzyme. Thus, water molecules present in a polymerization reaction may be more or less reactive depending on the degree by which the water molecule is associated with protein. Also, the rate at which water diffuses from the PPL enzyme particle into the reaction media is not known and is not readily determined since the polymerization media composition changes dynamically throughout the polymerization process. If eq 2 correctly predicts the measured M_n value, it can be used to determine the available water present which reacts to initiate chain growth (reacted water). Thus, assuming consumption of added butanol for chain initiation, it was estimated that of the 0.29 mmol of water present, 0.06 and 0.23 mmol of water reacted for chain initiation in reactions to form products **3** and **4**, respectively (see Table 1). Equation 3 can also be used to calculate the reacted water.

$$\text{reacted water (mmol)} = \text{mmol of polymer chains} \times \text{mol fraction of acid end groups} \quad (3)$$

In eq 3 the mmoles of polymer chains is determined from the monomer consumed and the product M_n. The mole fraction of acid end groups as well as M_n and conversion values were determined from ^{1}H NMR analyses as was described in the Experimental Section. The values obtained for reacted water using eq. 3 are tabulated in Table 1. Differences will arise in calculations of reacted water by eqs 2 and 3 if butanol is not completely consumed for chain initiation. Indeed, calculation of the reacted butanol for product **3** using eq 3 (where the mole fraction of butyl ester end groups is used in place of the mole fraction of acid end groups) showed that 0.24 mmol of the initial 0.30 mmol of butanol added was consumed for chain initiation. For products **4** and **5**, butanol appeared consumed by the same analysis. Inputting 0.24 mmol as the butanol concentration in eq 2 gives a value of reacted water of 0.12 mmol, which is identical to that calculated from eq 3 (see Table 1). Reacted water values calculated by eqs 2 and 3 for reactions to form products **4**–**6** are in excellent agreement (% error < 20%) while comparisons for products **7** and **8** were not possible since the percent conversions were not determined.

Scheme 1



As was anticipated, as the monomer/butanol ratio is increased at constant water concentration the mole fraction of acid end groups increases to a value of 0.86 for product **6**. Values for the additive mole fraction of butyl ester and acid end groups for products **3**, **4**, **5**, **6**, **7**, and **8** (see Table 1) were 0.98, 0.82, 0.86, 0.86 0.88, and 0.87, respectively. These results suggest that initiators other than butanol and water may be active in the polymerization reactions carried out to form products **4**–**8**. The fact that the PPL catalyst used herein is crude and contains only 25% by weight protein (see Experimental Section) may explain the origin of other molecules in these polymerization reactions that are capable of chain initiation. At present, it is not clear as to why product **3** appears to be formed with little to no initiation by molecules other than butanol or water.

In principle, based on the above and the use of purified PPL, if the quantity of water in the polymerization reaction is decreased to a value where the remaining water is tightly bound to the enzyme and has little to no reactivity, it should be possible to obtain products where the chains are initiated by only the specific initiator added. In this way, chain end structure can be specifically tailored as desired. Also, it is likely that high molecular weight PCL was not obtained in this work due to the presence of water in excess of that needed to maintain enzyme activity.

Polymerization Mechanism. The ^{1}H NMR analyses of PCL products given above indicate that enzyme-catalyzed ring opening of ϵ-CL takes place where butanol and water can act as initiators for chain growth. Analysis of PPL by others suggests that serine acts as the nucleophile for ester hydrolysis and that the serine residue is the catalytically essential region where it hydrolyzes the ester function by attacking the ester's carbonyl group.[19,20] From the above, the mechanism shown in Scheme 1 for PPL-catalyzed ϵ-CL polymerization is proposed.

In this work, the PCL products obtained had M_w/M_n values which are well above 1.0 (1.4–1.9; see Table I). A number of factors may contribute to this. For example, random degradative reactions between water and polymer chains, reactions such as alcoholysis, and enzyme-catalyzed transesterification may result in M_w/M_n values which deviate significantly from 1.0.

Further studies are in progress in our laboratory to better understand mechanistic features of enzyme-catalyzed lactone polymerization as well as to exploit this interesting new method for the preparation of novel polymer structures.

Summary of Results

In this study the effects of reaction temperature and solvent on the rate of conversion of ϵ-CL to PCL were evaluated. It was concluded that the polymerizations were suitably carried out in heptane at 65 °C. Polymer molecular weight increased slowly with conversion, suggesting that this is a chain reaction with rapid initiation and slow propagation. Increases in the ϵ-CL/butanol ratio from 15/1 up to where no butanol was added showed only modest increase in product molecular weight. This was explained by the fact that the water in polymerizations was active in chain initiation. Variation in the monomer/butanol ratio at constant water concentration resulted in PCL chains with from 0 to 0.65 mol fraction of butyl ester and from 0.33 to 0.86 mol fraction of carboxylic acid chain end groups (by ^{1}H NMR analyses). The presence of water concentrations in polymerization reactions above that which is strongly enzyme bound is believed to be an important factor which limited the formation of PCL chains of significantly higher molecular weight.

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