Enzyme-Catalyzed Stereoelective Ring-Opening Polymerization of α-Methyl-β-propiolactone

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ABSTRACT: The lipase-catalyzed stereoelective ring-opening polymerization of racemic α-methyl-β-propiolactone (MPL) was investigated. Using the lipase PS-30 from *Pseudomonas fluorescens*, a direct route to optically active (S)-enriched poly(α-methyl-β-propiolactone), PMPL, was demonstrated. From a comparative study of different organic media, polymerizations conducted in toluene and heptane proceeded more rapidly than those carried out in dioxane. The enantiomeric ratios *E* in toluene, heptane, and dioxane were 4.1 ± 0.2, 0.9, and 2.0, respectively. Thus, from the point of view of reaction rates and enantioselectivity, toluene was found to be the preferred solvent. PMPL products prepared in toluene by PS-30 catalysis had M_n values from 2600 to 2900 g/mol and $[\alpha]^{25}_D$ +12.2° to +19.0° (*c* 0.9 g/dL, $CHCl_3$). Analysis of the polymer chain end structure by ^1H and ^{13}C NMR showed that these products have hydroxyl and carboxylic acid termini. Based on the analysis of chain stereosequence distributions by ^{13}C NMR, it was concluded that stereoselectivity during propagation results from catalyst enantiomorphic-site control. Investigation of the thermal behavior of PMPL (75% (S)) by DSC showed that melting occurs over a broad region from ∼25 to 100 °C where the total ΔH_f is 12.7 cal/g.

Introduction

Pioneering work by Klibanov[1,2] and others[3−5] carried out on small molecules has quite elegantly demonstrated that suspensions of commercial enzymes in anhydrous organic solvents are powerful catalysts for a range of enantio- and regioselective transformations. From a review of this literature, it is clear that many opportunities are presented by exploiting enzyme technology to solve problems in enantio- and regioselective polymer synthesis and modification.

Investigations directed toward enzyme-catalyzed polyester synthesis have been reported by a few laboratories. Klibanov and co-workers reported the preparation of chiral oligoesters by enzyme-catalyzed transesterification between a racemic diester and an achiral diol or a racemic diol and an achiral diester.[6] This strategy for polyester synthesis was further developed by Wallace and Morrow.[7] These workers synthesized polyesters by enantioselective polymerization of bis(2,2,2-trichloroethyl) *trans*-3,4-epoxyadipate with 1,4-butanediol using porcine pancreatic lipase (PPL) as the catalyst (M_w = 7900 g/mol, >95% optical purity). Gutman and co-workers[8] reported the enzymatic synthesis of chiral oligoesters (ee ∼34%) from the prochiral substrate dimethyl β-hydroxyglutarate. Dordick and co-workers[9] prepared sucrose-containing polymers via a chemoenzymatic synthesis.

An interesting route to enzyme-catalyzed polymer forming reactions is that of ring-opening chemistry. Knani et al.[10] showed that ϵ-caprolactone (ϵ-CL) ring-opening polymerization catalyzed by PPL (624 h, 40 °C) leads to high monomer conversion and oligomeric poly(ϵ-caprolactone), PCL (M_n = 1900 g/mol). Kobayashi and co-workers[11] have reported the polymerization of γ-valerolactone and ϵ-CL. Of the lipases studied, that from *P. fluorescens* (PS-30) gave the highest percent ϵ-CL conversion (92%) and PCL molecular weight (M_n = 7700 g/mol; degree of polymerization, DP = 68). Recently, Kobayashi and co-workers synthesized polyesters from the macrolactones 11-undecanolide and 15-pentadecanolide.[12] Our laboratory has reported[13] on the effects of temperature, solvent, reaction water content, and ϵ-CL to initiator (butanol) ratio on the PPL-catalyzed rate of ϵ-CL conversion to PCL. The polymerizations were suitably carried out in heptane at 65 °C. The polymer molecular weight ranged from 1600 to 2700 g/mol where increased molecular weight was favored by decreased butanol and water concentrations. Also, the carboxylate chain ends were mixtures of butyl ester and carboxylic acid groups due either to chain hydrolysis or to competition between water and butanol as chain initiators.[13] Recently, we have found that an enzyme catalyzed lactone polymerization reaction met many of the requirements of Inoue's immortal polymerization of ϵ-CL initiated by the aluminum porphyrin system.[14]

In parallel with this study, work by us was performed to demonstrate a chemoenzymatic route to enantioenriched poly(α-methyl-β-propiolactone), PMPL.[15] This involved first carrying out an enzymatic resolution of α-methyl-β-propiolactone (MPL) followed by the polymerization of (*R*)-enriched MPL using the initiator CH_3CO_2K/dibenzo-18-crown-6. Of the enzymes investigated, PS-30 showed the highest stereoselectivity for the resolution of racemic MPL by methanolysis.[15] Also, even though the molar ratio of methanol to MPL was high (2 to 1), it was observed that resolutions catalyzed by PS-30 in hexane resulted in substantial quantities

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of oligomer. In other words, MPL ring-opened products appeared to be favorable nucleophilies for MPL ring-opening resolution. Previously published routes to optically active PMPL involved the chemical transformation of either (*R*)- or (*S*)-methyl 3-hydroxy-2-methylpropionate to the corresponding 3-bromo derivatives and subsequent ring closure to form enantioenriched MPL.[16] Since there was a loss of enantiopurity relative to the starting chiral synthon, the maximum MPL % (*R*) or (*S*) contents obtained were ~79%, whereas the use of PS-30-catalyzed resolution (55% conversion, diethyl ether) gave 93% (*R*)-MPL.[15]

Motivated by insights gained in the course of our study on MPL resolution,[15] work was undertaken herein to investigate whether, instead of carrying out a two-step process of monomer resolution and subsequent polymerization, it might be possible to directly obtain enantioenriched PMPL via a stereoelective polymerization catalyzed by PS-30. This took on additional interest since, to our knowledge, an enzyme-catalyzed stereoelective ring-opening polymerization had not as yet been documented. Previous attempts at stereoelective polymerization of racemic lactones have typically involved the use of chiral organometallic catalyst systems and have led to only modest enantiomeric enrichment of the final products.[17,18]

This paper describes the effects of experimental parameters on the kinetics and enantioselectivity of PS-30-catalyzed racemic MPL polymerization. The optically active polymers formed were characterized to determine the product molecular weight averages by gel permeation chromatography (GPC), thermal properties by differential scanning calorimetry (DSC), repeat unit sequence distribution by ^{13}C nuclear magnetic resonance (NMR), and end group structure by ^{1}H and ^{13}C NMR.

Experimental Section

Instrumental Methods. Proton (^{1}H) NMR spectra were recorded on a Bruker ARX-250 spectrometer at 250 MHz. ^{1}H NMR chemical shifts in parts per million (ppm) are reported downfield from 0.00 ppm using tetramethylsilane (TMS) as an internal reference. The concentrations used were 4% w/w in chloroform-*d* for polymer products and 3−5% w/w in deuterated solvents (see below) for monomer/polymer mixtures. The instrumental parameters were as follows: temperature 310 K, pulse width 4.9 μs (30°), 32K data points, 3.17-s aquisition time, 1-s relaxation delay, and 16 transients. Measurements of enantiomeric excess (ee) for MPL were carried out by using the chiral shift reagent europium(III) tris-[3-((heptafluoropropyl)hydroxymethylene)-(+)-camphorate], Eu-(+)-(hfc)$_3$ (40 mol % with respect to MPL). Solutions contained Eu-(+)-(hfc)$_3$ with either 0.5% w/w purified (by column chromatography; see below) MPL in chloroform-*d* or 1% w/w of a crude polymer/monomer mixture in chloroform-*d*/toluene-d_8, 3:1. The relative peak areas corresponding to the respective diasteriomeric complexes formed were determined by peak shape approximation using Gaussian curve fitting and then by cutting and weighing peaks. Carbon-13 (^{13}C) spectra were recorded at 62.9 MHz on a Bruker ARX-250 spectrometer in chloroform-*d* solutions, with chemical shifts in ppm referenced relative to $CDCl_3$ as the internal reference at 77.00 ppm. Polymer spectral aquisitions were conducted as 5.0% w/w $CDCl_3$ solutions using the following parameters: temperature 310 K, 30° pulse width, 64K data points, aquisition time 1.638 s, delay time 1 s, 15 000−20 000 transients. For improved resolution of diad and triad stereosequences, a Gaussian multiplication function with line broadening = −0.4 Hz/point and Gaussian broadening = 0.6 was used for data processing. Quantitation of signals corresponding to diad and triad sequences was carried out by peak shape approximation using Gaussian curve fitting and then by cutting and weighing peaks. For the COSY experiment (4% w/w polymer in $CDCl_3$), the data were collected in a 1024 × 128 data matrix and zero-filled to 512 × 512 using 8 scans per increment, a 1915 Hz sweep width, and 1.93 s delay between transients. The data were processed using sine-bell weighting.

Specific rotation measurements of PMPL ($[\alpha]^{25}_{589nm}$) were recorded on a Perkin-Elmer 241 polarimeter attached to a refrigerated constant temperature circulator using a 1.0-dm path length quartz microcell, temperature 25 °C, wavelength 589 nm (sodium D line), and PMPL concentration in chloroform of 0.90 g/dL (0.90, $CHCl_3$). Polymer molecular weight measurements by GPC and thermal characterization by DSC were carried out as described previously.[15]

Synthetic Procedures. Preparation of Racemic α-Methyl-β-propiolactone (MPL). The method was exactly as described by us elsewhere.[15] Prior to use of the monomer for polymerization reactions, it was fractionally distilled from CaH_2 under an argon bleed (46 °C, 4.5 mmHg) to give a colorless liquid. The ^{1}H NMR and IR spectra were consistent with that previously reported.[16]

Stereoelective Enzyme-Catalyzed MPL Polymerizations. Polymerizations were carried out in the following deuterated solvents: toluene-d_8, heptane-d_{16}, and dioxane-d_8 (% deuteration of at least 99% for each as specified by Aldrich). These solvents were distilled from CaH_2 under an argon atmosphere prior to use. The lipase from *Pseudomonos fluorescens* (PS-30) was obtained from Amano. Its activity defined as the nanomoles of *p*-nitrophenyl acetate (pNPA) hydrolyzed to *p*-nitrophenol (pNP) in dioxane per unit weight of enzyme per minute (nmol of pNP/(min·mg)) was measured by using pNPA and methanol as substrates in 1,4-dioxane and found to be 3.0. The concentration of the reaction product pNP was determined by absorbance measurements at λ_{max} (305 nm). See ref 15 for details on the method used for the determination of enzyme activity.

The following is a representative procedure used for carrying out the polymerization reactions. Into oven-dried reaction vials (20 mL) under an argon atmosphere were transferred 750-mg portions of PS-30 which had previously been dried in a vacuum dessicator (0.1 mmHg, 25 °C, 16 h). The vials were immediately stoppered with rubber septa and purged with argon. Subsequent reagents which included 5 mL of a dry deuterated solvent (toluene-d_8, heptane-d_{16}, or dioxane-d_8) and racemic MPL (0.5 g, 5.8 mmol) were added via syringe under argon. The reaction vials were placed into a shaker incubator (35 °C, 200 rpm) with reaction times of 48−744 h. Control reactions were set up as described above except PS-30 was not added. For monitoring the progress of the polymerizations approximately 0.2 mL of the reaction mixture was withdrawn via syringe under argon and filtered through a 0.45-μm filter to separate the enzyme catalyst from the product. The filtered enzyme was washed 2 times with 0.3-mL portions of $CDCl_3$. The filtrates were combined and analyzed by ^{1}H NMR to determine the monomer conversion to PMPL (see Instrumental Methods, above). For reactions carried out in toluene-d_8, Eu(hfc)$_3$ was added to crude monomer/polymer mixtures (see Instrumental Methods, above) which provided values from ^{1}H NMR measurements for the MPL ee as a function of % conversion. This approach did not prove useful for reactions conducted in heptane-d_{16} or dioxane-d_8 so that for these solvents, reactions were terminated and MPL enantiopurity was measured by ^{1}H NMR with Eu(hfc)$_3$ only at reaction end points where sufficient quantities of MPL were available for isolation. At the times specified below, the contents in the 20-mL reaction vials were subjected to vacuum filtration (glass-fritted filter, medium-pore porosity), insolubles were washed 3 times with 10-mL portions of dichloromethane, and the filtrates were combined and then divided into two portions. One portion was rotary evaporated (15 mmHg, 40 °C, 1 h) and then dried in a vacuum oven (2 mmHg, 65 °C, 48 h). The resulting PMPL was weighed to determine the monomer conversion and then analyzed by NMR, GPC, DSC, and polarimetry (see below). The second filtrate portion was concentrated by bubbling argon through the solution, and the monomer was then isolated from the crude mixture by silica

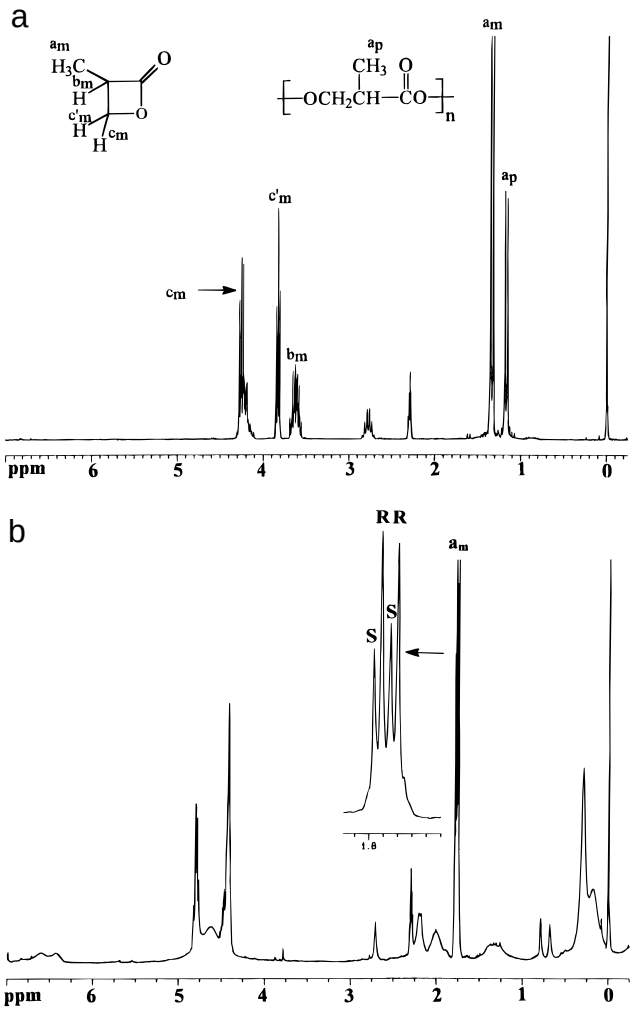


Figure 1. ^{1}H NMR (250-MHz) spectrum of the MPL/PMPL mixture in toluene-d_8/chloroform-*d* (1/3) before (a) and after (b) addition of 40% mol. Eu(+)-(hfc)$_3$ relative to MPL.

gel (Merck, 230−400 mesh, 60 Å) column chromatography. The chromatography was carried out by placing ~1 g of the crude reaction mixture on top of the column (25 cm long, 2-cm diameter) which contained 80 g of silica gel. Dichloromethane was used as the eluent, the flow rate was 2 mL/min, and MPL was eluted in volumes between 80 and 100 mL. Pure monomer fractions from column separation were stripped of solvent by rotory evaporation and then analyzed to determine the enantiopurity by ^{1}H NMR (see Instrumental Methods above).

Results and Discussion

Polymerizations of MPL catalyzed by PS-30 were carried out in heptane as well as deuterated toluene and dioxane. The use of deuterated reaction media provided a convenient method to determine monomer conversion as a function of reaction time. Thus, filtered reaction aliquots containing MPL/PMPL mixtures were analyzed directly by ^{1}H NMR spectroscopy to determine the monomer conversion (see Experimental Section for additional details). The spectrum of the monomer/polymer mixture resulting from a polymerization conducted in toluene-d_8 for 48 h is shown in Figure 1a. Peak assignments for MPL in Figure 1a are based on a previous report,[16] while assignments of PMPL ^{1}H NMR signals are discussed below (also, see Figure 3). The values of monomer conversion were obtained by the relative intensities of PMPL methyl (protons a_p, doublet at 1.15 ppm) and MPL methyl (protons a_m, doublet at 1.35 ppm) signals (see Figure 1a). The results of

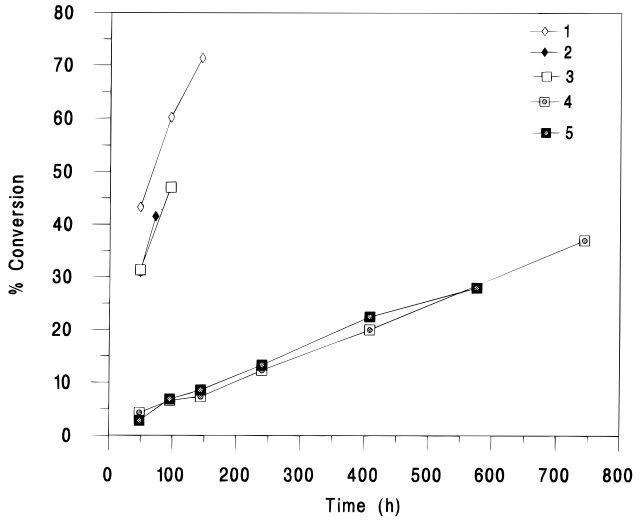


Figure 2. Plots of conversion of MPL as a function of time (curves 1−3 correspond to reactions carried out in toluene-d_8, curves 4 and 5 to reactions in dioxane-d_8).

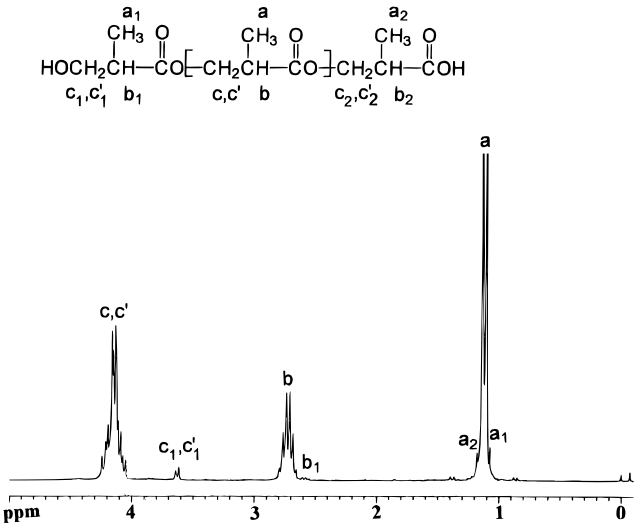


Figure 3. ^{1}H NMR (250-MHz) spectrum of PMPL obtained in toluene-d_8 (product T96-3, 47% conversion).

replicate experiments carried out in toluene-d_8 and dioxane-d_8 show that monomer conversion to PMPL proceeds at much faster rates in the former solvent (see Figure 2). For example, the % conversions measured after 96 h for reactions in toluene and dioxane were 47% and 7%, respectively. For polymerizations conducted in heptane, monomer polymerization resulted in aggregation of enzyme particles which likely occurred due to the low solubility of PMPL in this solvent. Thus, in heptane, MPL conversion was not followed during the course of reactions but was only obtained at the termination of the polymerization (see Experimental Section). Based on the results from the 144-h reaction times, the monomer conversions in heptane and toluene were similar (63% and 71%, respectively; see Table 1). Control reactions maintained under the same conditions as above but without the addition of enzyme did not show noticeable conversion of MPL. Thus, it is concluded that polymerizations proceeded via an enzymatically-catalyzed mechanism.

To determine the end group structure of PMPL chains formed by enzyme-catalyzed ring-opening polymerization, ^{1}H and ^{13}C NMR studies were carried out. The ^{1}H NMR spectrum along with peak assignments of the PMPL product T96-3 (see Table 1 footnotes for an

Table 1. Effect of Solvent and Conversion on PMPL Optical Purity

product[a]	solvent	conversion, %	MPL (unreacted) comp $[S/(R+S)]_m$	E	ee_m, MPL (unreacted)[b]	PMPL comp $[S/(R+S)]_p$[c]	ee_p, PMPL[b]	$[\alpha_0]^{25}_D$ PMPL,[d] g/dL
T144-1	toluene	71	0.08	4.2	0.84	0.67	0.34	+12.2
T96-3	toluene	47	0.28	4.7	0.45	0.75	0.50	+19.0
D744-1	dioxane	37	0.42	2.0	0.16	0.63	0.26	+7.2
H144-1	heptane	63	0.41	0.9	0.19	0.62	0.24	+4.3

[a] Letter in abbrevations correspond to the solvent used (T, toluene; D, dioxane; H, heptane) followed by polymerization time in hours and the experimental run. [b] Enantiomeric excess was determined as $ee_m = [(R-S)/(R+S)]_m$ for unreacted MPL and $ee_p = [(S-R)/(R+S)]_p$ for formed PMPL. [c] PMPL composition was calculated by equations $S_p = 0.5 - \{(1-C)[S/(S+R)]_m\}$ and $R_p = 0.5 - \{(1-C)[R/(S+R)]_m\}$, where C is conversion and $[S/(R+S)]_m$, and $[R/(S+R)]_m$ are the fractions of (S) and (R) enantiomers in unreacted MPL. [d] See Experimental Section for specific rotation measurement experimental parameters.

Table 2. Effect of Solvent on PMPL Molecular Weight

product	convsn,[a] % NMR	M_n,[b] NMR	M_n,[c] GPC	M_w/M_n[d]
T144-1	71	2600	2000	1.7
T72-2	41	2900	2200	1.8
T96-3	47	2700	2200	1.8
D744-1	37	600	500	1.3
D96-1	28	400	400	1.1
H144-1	63	2700	1900	1.8

[a] Determined by ^{1}H NMR measurements (see Figure 1a). [b] Determined by comparison of the spectral intensity for ^{1}H NMR signals at 3.63 ppm (protons c_1 and c_1') and 4.13 ppm (protons c and c′). [c,d] Determined by GPC analysis relative to polystyrene standards (see Experimental Section).

explanation of product abbreviations), which has an M_n by GPC of 2200 g/mol (see Table 2), is shown in Figure 3. Published chemical shift parameters for model compounds[19] predict an upfield shift by ~0.56 ppm for methylene protons c_1/c_1' relative to c/c′. Thus, the signals at 3.63 ppm were assigned to protons c_1/c_1'. A COSY experiment (see Experimental Section) performed on product T96-3 (spectrum not shown) provided the required information to confirm the assignment of signals due to protons in intrachain repeat units as well as at chain end positions. Most importantly, correlations were found for signals with peaks or midpoints at 3.63 with 2.60 ppm and 2.60 with 1.08 ppm. Thus, the signals at 2.60 and 1.08 ppm were assigned to protons b_1 and a_1, respectively. The assignment of the signal at 1.18 ppm to a_2 is based on the formation of methyl ester derivatives at carboxylic acid chain termini by reaction with diazomethane,[20] which resulted in an upfield shift of this signal. Further confirmation of assignments for signals corresponding to protons at chain hydroxyl termini (c_1/c_1', b_1, and a_1) was obtained by formation of the corresponding trifluoroacetate ester derivative,[21] which resulted in downfield shifts of signals formerly observed at 3.63, 2.60, and 1.08 ppm, respectively. Expanded regions of the 62.9-MHz ^{13}C NMR spectrum of PMPL T96-3 are shown in Figure 4. Assignment of intrachain repeat units follows as predicted by calculations using published chemical shift parameters,[19] while signals arising due to the effects of diad and triad stereochemical sequences were described in detail elsewhere.[15] Formation of the trifluoroacetate ester derivative at hydroxyl chain terminal repeat units[21] resulted in substantial downfield shifts for signals at 13.25, 41.94, 64.53, and 174.93 ppm which were therefore assigned to carbons 4a, 2a, 3a, and 1a, respectively. Modification of the carboxyl chain terminal repeat units by formation of the corresponding methyl ester derivative[20] resulted in upfield shifts in signals at 38.80 and 175.77 ppm which were therefore assigned to carbons 2b and 1b, respectively. The unassigned signal at 173.39 is likely due to carbonyl groups associated with penultimate repeat units. Since

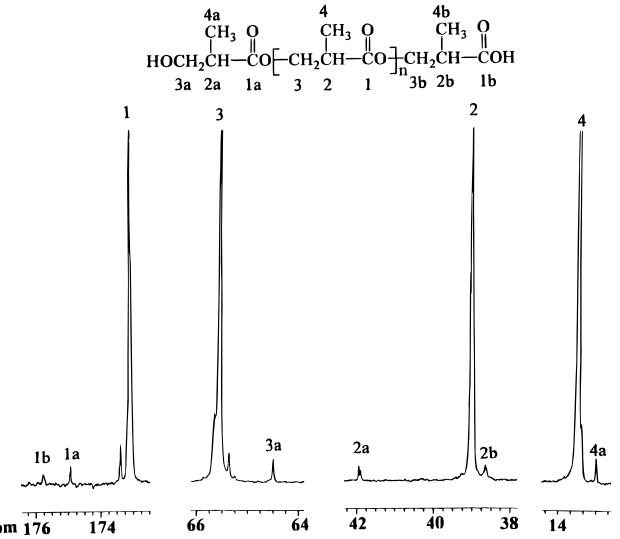


Figure 4. ^{13}C NMR (62.9-MHz) spectrum of PMPL obtained in toluene-d_8 (product T96-3, 47% conversion).

3b was insensitive to methylation of carboxyl terminal units, it likely gives rise to the signal at either 65.36 or 65.72, where additional complexity observed in this region may result from penultimate repeat units. It was concluded that the chains formed have chain end structures as shown in Figures 3 and 4 when the following are considered: (1) almost identical intensity of carbon signals 2a and 2b; (2) the observation of proton signals c_1/c_1', which when used to calculate molecular weight gave values in close agreement with GPC measurements (see Table 2); and (3) the observation of the carboxylic acid signal at 175.77 ppm and the intensity of the proton methyl ester signals formed by diazomethane derivatization. This is consistent with previous work on lipase-catalyzed ϵ-CL ring-opening polymerizations.[11,13]

Number-average (M_n) molecular weight values and polydispersities (M_w/M_n) of the synthesized polymers are shown in Table 2. For purposes of consistency, the values for M_n reported in the text below are from ^{1}H NMR measurements. PMPL products obtained using toluene as the organic media (T144-1, 71% conversion; T72-2, 42% conversion; T96-3, 47% conversion) had relatively similar M_n (2600, 2900, and 2700 g/mol, respectively) and M_w/M_n (1.7, 1.8, and 1.8, respectively) values. That M_n did not increase at higher conversion (71%) may be due to enzyme-catalyzed chain hydrolysis reactions. In other work by us, it was shown that chain cleavage due to water present in PPL-catalyzed ϵ-CL polymerizations does occur and was believed to be responsible for decreases in product molecular weight at high conversion where propogation rates decrease.[22] PMPL synthesized in heptane (H144-1) had similar M_n

and M_w/M_n values relative to those prepared in toluene (see Table 2). In contrast, the M_n values of products D744-1 and D576-2 (% conversions of 37 and 28, respectively) were relatively low (600 and 400 g/mol, respectively). Once again, this is rationalized as resulting from enzyme-catalyzed chain hydrolysis, which would be increasingly important for the relatively slower propogation rates and polymerization times in dioxane as opposed to toluene or heptane organic media (see above).

The enantioselectivity of PS-30-catalyzed MPL polymerizations was determined based on the knowledge of monomer conversion (see above) and the stereochemical purity of unreacted MPL. At the termination of polymerizations, the remaining MPL was purified by column chromatography (see Experimental Section). The optical purity of unreacted MPL was determined by ^{1}H NMR using Eu-(+)-(hfc)$_3$ as a chiral shift reagent. Complexation of MPL stereochemical mixtures with Eu-(+)-(hfc)$_3$ causes unequal downfield shifts of doublets corresponding to the methyl protons in (*S*)- and (*R*)-MPL isomers.[15] Relative integration values of the (*R*)- and (*S*)-MPL doublets were used to calculate the residual monomer enantiomeric composition ((*S*) mole fraction = [*S*]/{[*R*] + [*S*]}) and enantiomeric excess (ee, [*R*] − [*S*]/[*R*] + [*S*]) values. From information on unreacted monomer enantiomeric composition and conversion, the values for PMPL enantiomeric composition and ee were determined and are compiled in Table 1 (see Table 1 footnotes for equations used). As a strategy to circumvent the need for separation of MPL from reaction mixtures prior to ^{1}H NMR ee measurements, attempts were made to directly analyze withdrawn reaction aliquots. While this was not possible for reactions in dioxane-d_8 due, presumably, to strong chelation by the solvent to Eu-(+)-(hfc)$_3$, favorable results were obtained for reactions conducted in toluene-d_8. In Figure 1b, a ^{1}H NMR spectrum recorded using 40 mol % Eu-(+)-(hfc)$_3$ (relative to MPL) in toluene-d_8/chloroform-*d* (1/3) of the crude monomer/polymer mixture T48-2 (31% conversion) is shown. Expansion of the methyl proton region in Figure 1b shows the extent by which signals corresponding to (*R*)- and (*S*)-MPL were resolved. Since baseline resolution was not achieved, the stereochemical composition of MPL in mixtures with PMPL was obtained by peak shape approximation of the partially resolved (*R*)- and (*S*)-MPL methyl proton signals by Gaussian curve fitting (see Experimental Section). For quantitative determination of reaction enantioselectivity as a function of the organic solvent used, the values of the enantiomeric ratio *E* were calculated using eq 1 derived by Sih and coworkers:[23]

$$E = \frac{V_{\mathrm{max}A}/K_A}{V_{\mathrm{max}b}/K_B} = \frac{\ln[(1-c)(1-\mathrm{ee}_s)]}{\ln[(1-c)(1+\mathrm{ee}_s)]} \qquad (1)$$

where A and B are the fast- and slow-reacting enantiomers ((*S*)- and (*R*)-MPL, respectively), *c* is the conversion ($C_{\mathrm{initial}} - C_{\mathrm{final}}/C_{\mathrm{initial}}$, *C* is the MPL concentration), ee_s is the enantiomeric excess of the recovered substrate, and V_{max} and *K* are the maximal reaction rate and Mechaelis constant, respectively. Use of eq 1 assumes that (*R*)- and (*S*)-MPL compete for the same site of the enzyme, conversion of MPL to product is virtually irreversible, and there is no product inhibition. Figure 5 shows experimental conversion vs ee data points (denoted as inverted triangle) and a theoretical curve generated using eq 1 for MPL resolution via PS-30-catalyzed polymerization carried out in toluene-d_8. Since the theoretical curve is in close agreement with the experimental data points, it is reasonable to assume that this polymerization occurs by a mechanism that agrees with the assumptions used for the derivation of eq 1 (see above and ref 23). The *E* value found by curve fitting is 4.1 ± 0.2. The *E* values determined based on ee_s and conversion results at reaction end points using dioxane and heptane as solvents were 2.8 and 0.9, respectively. Thus, considering both polymerization reaction rates (Figure 2) and enantioselectivity (Table 1), toluene was found to be a preferred solvent for preparing PMPL enriched in the (*S*) enantiomer.

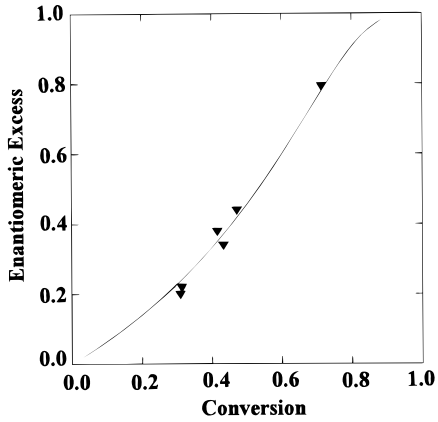
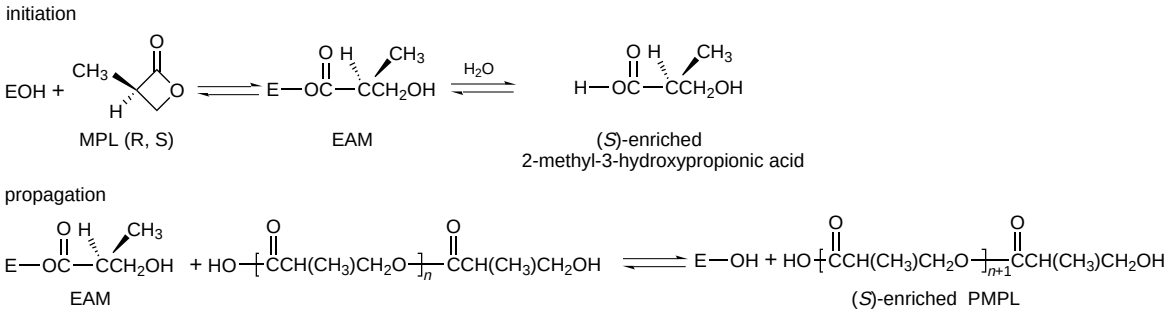


Figure 5. Experimental ee vs % conversion results (denoted as inverted triangles) for a polymerization carried out in toluene-d_8 along with a theoretical curve generated using eq 1.[23]

Optical rotation measurements (see Experimental Section) allowed determination of $[\alpha]^{25}_{589\mathrm{nm}}$ (*c* 0.90, $CHCl_3$) values which are listed in Table 1 for selected PMPL products. The $[\alpha]^{25}_{589\mathrm{nm}}$ values for products T144-1, T96-3, and H144-1 are +12.2°, +19.0°, and +4.3°, respectively. In another study by Xu et al.,[15] a linear relationship was found for a plot of PMPL $[\alpha]^{25}_{589\mathrm{nm}}$ (*c* 0.80, $CHCl_3$) vs % enantiopurity, indicating that measured PMPL specific rotation values result from the additive contribution of chain enantiomeric repeat units and are not substantially affected by stereosequence effects. The extrapolated value for 100% (*S*)-PMPL was +38.4°, and those predicted by linear regression for 67% and 75% (*S*)-PMPL were 14.0° and 19.9°, respectively. Although the PMPL products synthesized by Xu et al.[15] were of substantially higher molecular weight than those prepared in this work (M_n ~10 000 vs 2700 g/mol), the high specific rotation values of products T144-1 and T96-3 (12.2° and 19.0°, respectively) correspond well to those predicted by Xu et al.[15] based on chain enantiopurity. Thus, the optical rotation values measured herein support chain stereochemical compositional measurements and that high enantioselectivity was indeed achieved.

The thermal behavior of product T96-3 (75% (*S*)) was studied by DSC. The thermogram corresponding to the first heating scan (thermogram a, Figure 6) shows three distinct melting transitions with endotherm peak values (T_m) at 37, 66, and 89 °C. Thus, melting occurs over a broad region from ~25 to 100 °C where the total ΔH_f (over the entire melting region) is 12.7 cal/g. After quenching rapidly from the melt to obtain an amorphous sample, a second heating scan was carried out and is shown in thermogram b (see Figure 6). The second heating scan shows a T_g at −29 °C and that crystallization of the sample occurs (crystallization exotherm

Scheme 1



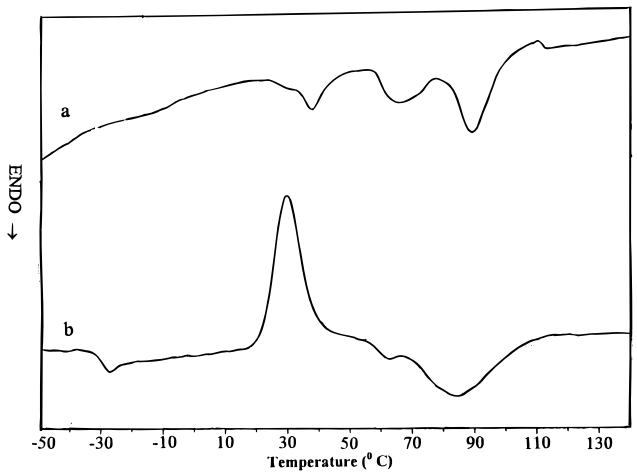


Figure 6. DSC thermograms recorded for PMPL (product T96-3, 47% conversion) from (a) the first heating scan and (b) the second heating scan after rapid quenching from the melt.

maximum, T_c, 29 °C) rapidly while heating above T_g. It is interesting to compare the thermogram of this sample to a PMPL copolyester containing 74% (*R*) repeat units (M_n 10 600, M_w/M_n = 1.25) which was prepared by polymerization of 74% (*R*)-MPL using CH_3CO_2K/dibenzo-18-crown-6 (1:2) as the initiator.[15] The latter polymer also has a broad melting transition (30−90 °C), four distinct melting temperatures (T_m values of 40, 62, 77, and 83 °C), a cumulative ΔH_f value of 14.0 cal/g, and a T_g of −25 °C measured on an amorphous sample quenched from the melt. The most dominant of the transitions was at 77 °C, whereas for product T96-3, the transition at 89 °C was the largest. In general, these two products which have almost identical stereochemical purities show similar thermal behavior. Observed differences in melting behavior are expected since the polymers differ in molecular weight, repeat unit stereochemical sequence distribution (see below and ref 15), and sample history.

An analysis of enzyme-polymerized PMPL repeat unit sequence distribution was undertaken. Previous work by Xu et al.[15] showed ^{13}C NMR spectra and expansions of the PMPL carbonyl, methyl, and methine carbon regions that allowed determination of the relative fractions of diad, diad, and triad repeat unit sequences, respectively. The experimental results obtained for products T144-1 and T96-3 diad and triad fractions are shown in Table 3. The triad distribution calculated from experimental diad intensities using the Bernoulli model[25] deviated significantly from experimental values where *is* + *si* and *ss* were substantially higher and lower, respectively (see Table 3). Also, the Bernoulli model triad test where $B = 4iiss/(si + is)^2 = 1$ led to calculated *B* values for T144-1 and T96-3 of 2.68 and 2.77, respectively, which are significantly higher than 1 for

Table 3. Diad and Triad Stereosequence Distributions and Statistical Analyses for PMPL Samples

product	PMPL $S/(S+R)$	diad fract[a] *i*	diad fract[a] *s*	triad fract[d] *ii*	triad fract[d] *ss*	triad fract[d] *is* + *si*	B[e]	E[f]
T144-1	0.67	0.67[b]	0.33	0.40	0.23	0.37		
		0.64[c]	0.36	(0.40)[g]	(0.12)	(0.48)	2.68	
				[0.46][h]	[0.18]	[0.36]		1.24
T96-3	0.75	0.74	0.26	0.50	0.16	0.34		
		0.69	0.31	(0.48)	(0.10)	(0.42)	2.77	
				[0.53]	[0.16]	[0.31]		0.94

[a] Given as the fraction of (*i*) isotactic and (*s*) syndiotactic diads as determined by ^{13}C NMR. [b] From carbonyl carbon resonance. [c] From methyl carbon resonance. [d] Given as a fraction of *ii*, *ss*, and *is* + *si* triads as determined by ^{13}C NMR from carbon methine resonance. [e] The Bernoulli model triad test, where $B = 4iiss/(is + si)^2$, such that $B = 1$ for ideal random propagation. [f] The enantiomorphic-site model triad test, where $E = 2ss/(is + si)$, such that $E = 1$ for perfect catalyst control. [g] The numbers in parentheses are the calculated triad stereosequences from the experimentally determined diad stereosequence values by using the following Bernoullian equations: $ii = i^2$, $ss = s^2$, and $is + si = 2si$. [h] The numbers in brackets are the calculated triad stereosequences from the experimentally determined diad stereosequence values by using corresponding relations between triad/diad sequences for enantiomorphic site model:[25,26] $ii = 1 - 3s/2$, $is + si = s$, and $2ss = s$.

ideal random chain propagation.[24] Interestingly, calculation of triad stereosequences from experimental diad fractions using the enantiomorphic-site model (see Table 3 legend *h*, and refs 25−27) resulted in better agreement between experimental and calculated triad fractions compared to the Bernoulli model. Furthermore, calculation of the test parameter $E = 2ss/(is + si)$ based on the enantiomorphic site model[28] gave values for T144-1 and T96-3 of 1.24 and 0.94, which is in good agreement with the theoretically predicted value of 1.0. Indeed, isospecific polymerization controlled by catalyst chirality usually follows the enantiomorphic-site control model.[26] This model assumes that catalyst structure and not the structure of polymer chain end controls the stereochemical configuration of the corresponding polymer. Also, this model assumes that the catalyst structure remains unchanged prior to each new monomer propagation step. Agreement with the enantiomorphic-site control model is consistent with our expectations for PS-30-catalyzed lactone polymerization where the enzyme reacts more rapidly with (*S*)- relative to (*R*)-MPL, forming an enzyme-activated monomer (EAM) complex which than reacts with a growing PMPL chain end. The stereochemistry of the added monomer is controlled by PS-30 during formation of the PS-30−MPL EAM complex. Furthermore, since no other initiator species was added to the polymerizations and PMPL chains were formed having hydroxyl and carboxylic chain ends (see above), it is presumed that water functioned as the nucleophile for chain initiation. Based on the above, a proposed mechanism for PS-30-catalyzed

MPL polymerization is shown in the Scheme 1. It is consistent with the model proposed by MacDonald et al.[13] and Uyama et al.[12] for the lipase-catalyzed polymerization of achiral lactones.

It is important to note that in the proposed model, it is assumed that the serine residue is the catalyticaly essential region where the enzyme hydrolyzes the ester functionality by attacking the ester's carbonyl group.[29] This is generally accepted for lipases which catalyze the hydrolysis of triglycerides.[29]

Summary of Results

Polymers were formed by PS-30-catalyzed ring-opening polymerization where the (*S*)-MPL enantiomer reacted more rapidly than its (*R*)-antipode. Thus, a direct route from racemic MPL to (*S*)-enriched (up to 75% (*S*)) PMPL was achieved. PMPL stereochemistry was determined from the stereochemical composition of residual monomer by ^{1}H NMR using the chiral shift reagent Eu-(+)-(hfc)$_3$. Toluene was found to be the preferred solvent relative to heptane and dioxane when considering the rates of monomer conversion, product molecular weight, and enantioselectivity. Based on the models developed by Sih and co-workers, a plot of ee vs conversion was constructed, and the enantiomeric ratio *E* in toluene thereby determined was 4.1 ± 0.2. PMPL products in toluene had M_n values up to 2900 g/mol and M_w/M_n values ranging from 1.7 to 1.8. Optical rotation values measured for optically active PMPL products formed herein supported that high chain enantiopurity was indeed achieved. As was expected for enzyme catalysis, analysis of the chain stereosequence distribution by ^{13}C NMR showed that the polymerization fit the enantiomorphic-site control model. The PMPL products formed were semicrystalline and melted over a broad temperature range. The major melting endotherm had a T_m of 89 °C. A polymerization mechanism was proposed that involved the formation (preferably with (*S*)-MPL) of an enzyme-activated monomer complex, initiation by water, and ring-opening propogation resulting in chains having hydroxyl and carboxylic acid chain termini.

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