

Mild, Solvent-Free ω -Hydroxy Acid Polycondensations Catalyzed by *Candida antarctica* Lipase B

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Immobilized *Candida antarctica* Lipase B (Novozyme-435) was studied for bulk polyesterifications of linear aliphatic hydroxyacids of variable chain length. The products formed were not fractionated by precipitation. The relative reactivity of the hydroxyacids was 16-hydroxyhexadecanoic acid \approx 12-hydroxydodecanoic acid \approx 10-hydroxydecanoic acid ($DP_{\text{avg}} \approx 120$, $M_w/M_n \leq 1.5$, 48 h, 90 °C) > 6-hydroxyhexanoic acid ($DP_{\text{avg}} \approx 80$, $M_w/M_n \leq 1.5$, 48 h, 90 °C). Remarkable improvements in molecular-weight buildup resulted from leaving water in the reaction. By 4 h, without application of vacuum, the DP_{avg} for 12- and 16-carbon hydroxyacids was about 90. In contrast, with identical substrates and water removal, the DP_{avg} at 4 h was about 23. Large differences in the molecular-weight build up of 12-hydroxydodecanoic acid were observed for catalyst concentrations (%-by-wt relative to monomer) of 0.1, 0.5, 1, and 10. Nevertheless, by 24 h, with 1% catalyst containing 0.1% lipase, poly(12-hydroxydodecanoic acid) with M_n 17 600 was formed. For 12-hydroxydodecanoic acid polymerization at 90 °C, the catalyst activity decreased by 7, 18, and 25% at reaction times of 4, 24, and 48 h, respectively. Furthermore, the retention of catalyst activity was invariable as a function of the substrates used.

Introduction

Isolated lipases are currently under study as catalysts for polymer synthesis in vitro.¹ Motivations for using lipases as catalysts include (1) promising substrate-conversion efficiencies for nonnatural substrates, (2) high enantio- and regioselectivity, (3) catalyst recyclability, (4) effective functionality in bulk reaction media (thus avoiding organic solvents), and (5) lack of toxicity.^{1a}

Normally, condensation polyesterifications are performed by ester-interchange reactions or by direct esterification of hydroxyacids or diacid/diol combinations.² However, using chemical catalysts for these reactions requires harsh conditions (e.g., temperatures > 200 °C) and metal catalysts potentially problematic for certain product end uses.³ These conditions can limit product molecular weight and reduce available building blocks, as many are unstable at such temperatures. For example, the condensation polymerization of 2-allylpropane-1,3-diol with adipic acid, catalyzed by $Ti(O^iPr)_4$ (220 °C under nitrogen), produced a yellow gel, suggesting that side-reactions and decomposition occurred.⁴ In contrast, lipase-catalyzed condensation polymerizations are metal free and function at moderate temperatures (see below).

Previous studies of these polyesterifications have focused primarily on reactions between diols and activated diacids.⁵ However, diacid activation is expensive with such groups, limiting the method's potential technological impact. Although important progress has been made in lipase-catalyzed

copolymerizations of acid and alcohol building blocks using nonactivated free acids,^{6–12} problems that must be overcome for practical use include long reaction times^{6,10} and excessive quantities of lipase.^{6,8,10}

Recently, we reported on catalysis of condensation polymerizations between adipic acid and 1,8-octanediol by immobilized Lipase B from *Candida antarctica* (CALB).¹² The effects of CALB concentration, reaction temperature, and choice of resin for CALB immobilization on the time-course of chain growth and polydispersity were studied. With CALB loading on the macroporous resin Accurel optimized, the activity of CALB on Accurel was found to be similar to that on Lewatit (Novozyme-435). Nonimmobilized CALB also catalyzed the polymerization but at a slower rate than immobilized CALB. The extent of chain growth was nearly invariable for reaction temperatures from 65 to 90 °C. We observed a similar lack of sensitivity over an even broader range of polymerization temperatures (20–108 °C) in previous studies of CALB-catalyzed lactone polymerizations.¹³

Our laboratory also reported on the effects of substrates and solvents on lipase-catalyzed-step-condensation polymerizations of diacids and diols.¹⁴ Diphenyl ether was the preferred solvent for the preparation of poly(octanedioladipate) (M_n 28 500, 48 h, 70 °C). Reactions with longer chain-length diacids (sebacic and adipic acid) and diols (1,8-octane and 1,6-hexane diol) provided higher reactivity than systems with shorter chain-length diacids (succinic and glutaric) and 1,4-butanediol. Furthermore, at 70 °C, the retention of catalyst activity, invariable as a function of the substrates

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used, was higher when reactions were conducted in diphenyl ether than in bulk.

To our knowledge, there are three reports on lipase-catalyzed direct condensations of linear aliphatic hydroxyacids. O'Hagan and Zaidi¹⁵ used a 10 times-by-wt excess of porcine pancreatic lipase (PPL) relative to 10-hydroxydecanoic acid to obtain the corresponding polymer with M_n 9346 and M_w/M_n 1.29 (55 °C, 48 h, in hexane, 60% product yield). In a separate paper,¹⁶ they reported that the polymerization of 11-hydroxyundecanoic acid by PPL (10/1 catalyst/monomer) gave a polymer of M_w 22 430 g/mol and M_w/M_n 1.2 (55 °C, 7 days, in hexane, yield not reported). Likewise, Shuai et al.¹⁷ reported that the PPL-catalyzed polymerization of 12-hydroxydodecanoic acid (2:1 w/w catalyst to monomer) formed a product with $M_w \leq 2900$ and $M_w/M_n \approx 1.25$ (75 °C, 56 h, in toluene). In the same publication, they reported on polymerization of 3-hydroxybutyric acid, but no product of significant molecular weight was formed ($M_w \leq 660$, $M_w/M_n \approx 1.2$). So although these references^{15–17} demonstrate the feasibility of lipase-catalyzed polyesterification of hydroxyacids, they describe reactions that are slow,^{16,17} use excessive quantities of lipase,^{15–17} and include solvent.^{15–17} Furthermore, opportunities to explore polydispersities for these systems have been missed because of product fractionation prior to molecular-weight analysis.^{15–17} Moreover, the importance of water on the reaction progress has been overlooked.

This paper describes bulk polyesterifications of linear aliphatic hydroxyacids catalyzed by immobilized *Candida antarctica* Lipase B (Novozyme-435). Work was conducted to (1) assess the progress of polymerizations of hydroxyacids over time, (2) compare polymerizations conducted with and without water removal under identical reaction conditions, (3) measure product molecular-weight averages and polydispersity, and (4) perform the above with substrates that differ in structure. The hydroxyacids studied include 6-hydroxyhexanoic acid, 10-hydroxydecanoic acid, 12-hydroxydodecanoic acid, and 16-hydroxyhexadecanoic acid. The effect of CALB concentration over ranges from 1 to 0.01% (by wt) was studied, and the retention of Novozyme-435 activity as a function of reaction time and substrate chain length is reported.

Experimental Section

Materials. 6-Hydroxyhexanoic acid, 10-hydroxydecanoic acid, 12-hydroxydodecanoic acid, and 16-hydroxyhexadecanoic acid were purchased from Aldrich Chemical Co. and were used as received. Novozyme-435 (specific activity 7000 PLU/g) was provided by Novozymes (Denmark) and consists of 10%-by-wt CALB physically adsorbed within 90%-by-wt Lewatit VPOC 1600 (supplied by Bayer). Lewatit is a macroporous resin comprised of cross-linked poly(methyl methacrylate-*co*-butyl methacrylate) with a surface area and average pore diameter of 110–150 m²g⁻¹ and 140–170 Å, respectively.¹⁸ CALB is found on the outer 100 μ m of 600 μ m average-diameter Lewatit beads.¹⁸ All chemicals were obtained in the highest possible purities available.

General Procedure for Lipase-Catalyzed Polyesterification of Linear Aliphatic Hydroxyacids. Novozyme-435

(10%-by-wt relative to total weight of monomer) dried in a vacuum desiccator (0.1 mm Hg, 25 °C, 24 h) was transferred to a round-bottom flask containing the monomer (20 mL flask, 600 mg hydroxyacid). The reactions were performed in bulk, and the flasks were capped with a rubber septum. The reaction flasks were then placed into a constant preset-temperature oil bath on a magnetic stirrer (IKA Werke, Rct Basic) at 220 rpm for a predetermined time. A vacuum (10 mm of Hg) was applied to remove water. Aliquots (about 3 mg) were removed from the reaction mixture at selected time intervals to monitor the reaction progress. The reactions were terminated by adding excess cold chloroform, stirring for 15 min, and removing the enzyme by filtration (glass-fritted filter, medium porosity). These nonfractionated products (no precipitation) were characterized by ¹H NMR and GPC (see below) to determine their molecular-weight distribution and to analyze the different species generated. Studies on the effect of catalyst concentration were performed with 12-hydroxydodecanoic acid as above but with 3 g instead of 600 mg substrate.

Assay Protocol for Lipase in Organic Media. The lipase activity in organic media was determined by the lipase-catalyzed esterification of lauric acid with propanol. The method used was identical to that described elsewhere.¹⁴

Other Instrumental Methods. *Nuclear Magnetic Resonance (NMR).* The polyesters formed by lipase-catalyzed polyesterifications of linear hydroxyacids were characterized using ¹H NMR spectra recorded on a Bruker NMR spectrometer (Model DPX300) at 300 MHz. The chemical shifts in parts per million (ppm) for ¹H NMR spectra were referenced relative to tetramethylsilane (TMS, 0.00 ppm) as the internal reference.

Gel Permeation Chromatography (GPC). Molecular weights were determined by GPC using a Waters HPLC system, details of which are described elsewhere.^{12,14} Chloroform was the eluent at a 1.0 mL/min flow rate. Sample concentrations of 0.2wt %/vol and injection volumes of 100 μ L were used. Molecular weights were determined based on a conventional calibration curve generated by narrow-molecular-weight polystyrene standards obtained from Aldrich chemical company.

The relative formation of different molecular-weight species in Figure 2a was determined by assuming a Bernoulli distribution for overlapping peaks and by cutting and weighing curve areas. Peak molecular weights in Figure 2a were determined based on polystyrene standards. These peak molecular weights were used to identify discrete regions of GPC traces.

Reaction Water Content. The water (wt %) in reactions was measured by using an Aqua star C 3000 titrator with Coulomat A and Coulomat C from EM-science. The water (wt/wt) in the reaction mixtures was determined by adding the sample (3 mg) in Coulomat A, stirring it in a closed septum container, and titrating against Coulomat C.

Results and Discussion

The rationale for the choice of Novozyme-435 as the catalyst was described elsewhere.¹² Novozyme-435 consists

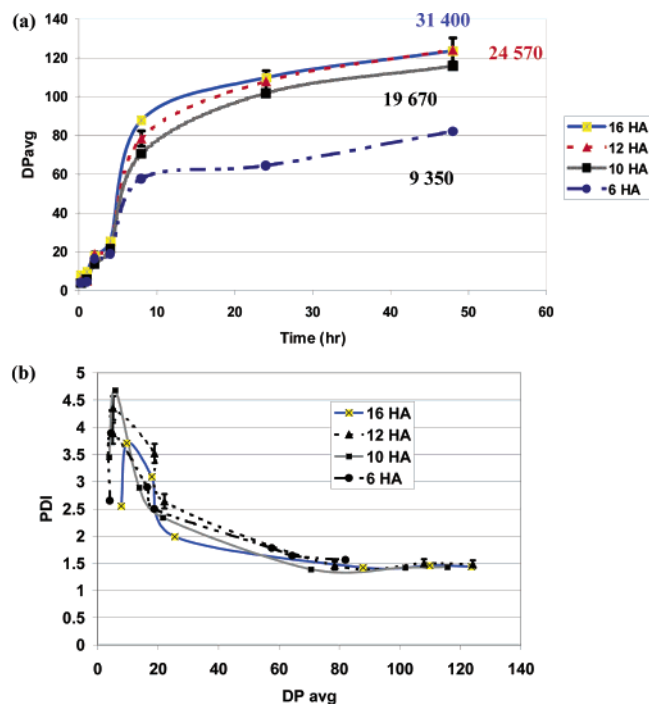


Figure 1. Lipase-catalyzed polyesterification of linear aliphatic ω -hydroxyacids (HA) (90 °C, in bulk, 10wt %/wt catalyst:monomer substrates, with application of vacuum): (a) extent of chain growth as a function of time (b) the polydispersity index (PDI) of products formed. The error bars were calculated from triplicate runs.

of *Candida antarctica* Lipase B (CALB) physisorbed to Lewatit, a macroporous polyacrylate resin (see the Experimental Section). Because previous work in our laboratory demonstrated the feasibility of bulk polymerizations of unactivated diacids and diols,^{12,14} solvent-less conditions were used throughout this study. A polymerization temperature of 90 °C was selected because it was sufficiently above the melting point of the monomers used. Higher temperatures were avoided because ϵ -caprolactone ring-opening polymerizations performed above 90 °C give decreased monomer conversion, presumably because of protein denaturation.¹⁹

Effect of Hydroxyacid Chain Length on DP_{avg} and PDI.

Hydroxyacids of different chain lengths were used as substrates for Novozyme-435-catalyzed bulk polymerizations (48 h, in vacuo). Figure 1a shows that by 4 h, regardless of the substrate chain length, all products had similar DP_{avg} values (about 20). However, by 8 h, the relatively slower progress of the 6-hydroxyhexanoic acid polymerization was evident. By 48 h, the DP_{avg} and M_w/M_n of poly(6-hydroxyhexanoate) were 80 and 1.5, respectively. In contrast, by 48 h, the polymerizations of 16-, 12-, and 10-carbon-chain-length ω -hydroxyacids gave products with DP_{avg} from 115 to 125 and M_w/M_n of 1.5. For example, by 48 h, poly(16-hydroxyhexanoate) was formed with M_n and M_w/M_n of 31 400 and 1.5, respectively. Thus, chain growth catalyzed by Novozyme-435 was similar for ω -hydroxyacids with chain lengths between C10 and C16 but was slower for C6. The difference in chain growth between C10 and C6 is consistent with the lipases' expected preference for substrates with chain lengths similar to the fatty acids of triglycerides. However, the invariability of the polymerizations for ω -hydroxyacids with chain lengths between C10 and C16 was

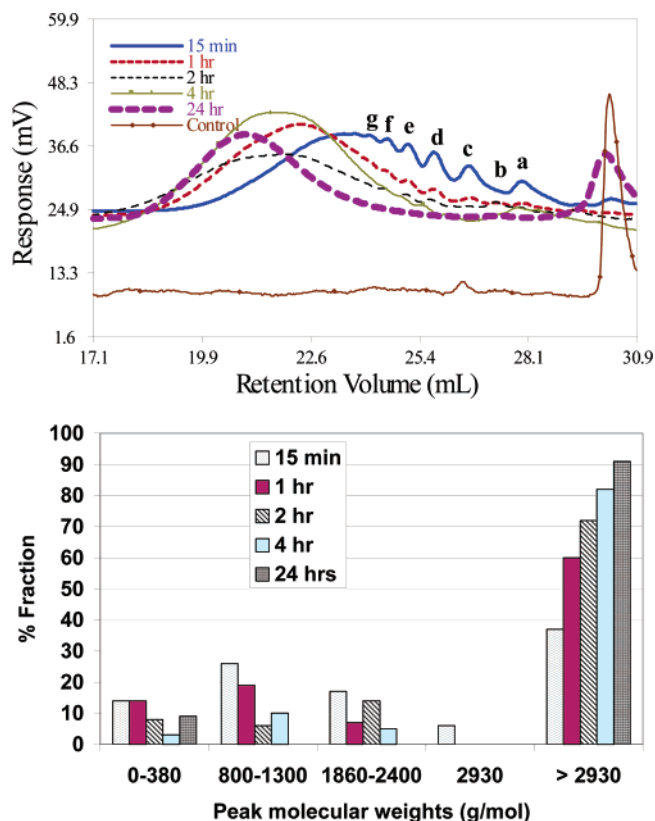


Figure 2. (a) GPC traces after different reaction times for the condensation polymerization of 12-hydroxydodecanoic acid (90 °C, in bulk, 10wt %/wt catalyst:monomer, with application of vacuum). The peak molecular weights are represented as a = 190 g/mol, b = 380 g/mol, c = 800 g/mol, d = 1 300 g/mol, e = 1 860 g/mol, f = 2 400 g/mol, g = 2 930 g/mol. (b) The %-by-wt relative to total sample for selected molecular species found in the product. Results are from the analysis of GPC traces displayed in Figure 2a.

surprising. For instance, studies of fatty-acid specificity of *Chromobacterium viscosum* lipase (CVL) on transesterification of fatty-acid methyl esters with 1-propanol showed that increasing the fatty-acid chain length (C₆ to C₁₈) increases the rate-constant parameter.²⁰ The rate constants of fatty acids from chain length C₆ to C₁₈ were as follows: 0.011 h⁻¹, C₆; 0.05 h⁻¹, C₁₀; 0.13 h⁻¹, C₁₂; 0.20 h⁻¹, C₁₄; 0.32 h⁻¹, C₁₆; 0.38 h⁻¹, C₁₈. Thus, as is normally found for lipases, the CVL lipase was sensitive to changes in chain length from C₆ to C₁₀ as well as from C₁₀ to C₁₆. Also, in a previous report, we showed a propagation rate 13 times more rapid for polymerizations of ω -polypentadecalatone (C₁₅) than ϵ -caprolactone (C₆) by using Novozyme-435 as the catalyst.²¹ Although comparison of lipase preferences of substrates with transesterification and lactone polymerization provides insight into substrate preferences, the chain-length effects for substrates participating in condensation polymerizations may differ due to differences in the mechanism of these reactions.

The subtlety of substrate-chain-length effects is illustrated by comparing the above results with ω -hydroxyacids to those for bulk step-condensation polymerizations of diacids/diols. Copolymerizations of 1,8-octanediol with either sebacic (C₁₀) or adipic (C₆) diacids at 70 °C occurred with no significant difference in DP_{avg} as a function of time.¹⁴ Thus, during step-condensation polymerizations, the enzyme did

not “feel” the difference between C-6 and C-10 diacids. Because the same enzyme did respond differently to C-6 and C-10 ω -hydroxyacids, it seems that the dissimilarities between chain length effects for diacid/diol and ω -hydroxyacids are complex and not readily predictable. Previous literature on lipase-catalyzed polymerizations of ω -hydroxyacids provides little guidance on substrate-chain-length effects. Studies by O’Hagan and Zaidi^{15,16} did not address the effect of substrate chain length. Shuai et al.¹⁷ reported more rapid molecular-weight build up for the polymerization of 12-hydroxydodecanoic acid relative to 3-hydroxybutyric acid, but these substrates have primary and secondary hydroxyl groups.

The PDI values in Figure 1b were determined on products not precipitated or fractionated prior to analysis. The figure shows the polydispersity index (PDI) increasing early in the polymerizations, when the DP_{avg} is ≤ 10 , to between 2.4 and 4.5. However, as the polymerizations progressed to $DP_{\text{avg}} > 10$, the PDI decreased to about 1.5 (standard deviation ± 0.1).²² Dispersities of 1.5 were similarly observed in previous studies of Novozyme-435-catalyzed condensations between diols and diacids.^{12,14} The PDI of polymers formed by Novozyme-435-catalyzed step-condensations are well below what is expected for analogous polymerizations catalyzed by traditional chemical catalysts, which lead to PDI values of 2 and above due to random statistical events of chain growth and transesterification.² In contrast, the results for lipase-catalyzed condensation polymerizations suggest that chain growth occurs with “chain-length” selectivity, with the lipase reacting at different rates for building blocks of different chain lengths. This results in products with relatively higher chain-length uniformity. The reports by O’Hagan and Zaidi^{15,16} and Shuai et al.¹⁷ show that polymers formed by polymerization of hydroxyacid have polydispersities ≤ 1.29 , well below 2.0. However, because the products were fractionated by precipitation prior to molecular-weight analysis, we cannot conclude that the origin of the narrow dispersity products was lipase selectivity.

Novozyme-435-catalyzed polymerizations of lactones such as ϵ -caprolactone and ω -pendacalactone give products with M_w/M_n values of about 2.0.^{19,21} Fundamental differences in the polymerization mechanism (e.g., step-condensation versus chain polymerization) may explain the disparity in product polydispersity.¹³ For instance, enzyme-catalyzed lactone ring-opening polymerization to about 75% conversion occurs by propagation reactions between enzyme-activated lactones and the hydroxyl terminus of chains. However, in step-condensation polymerizations, in place of lactones the acylating agent is enzyme-activated acid terminated chain segments. Furthermore, transesterification is known to occur during lactone-ring-opening polymerizations. Transesterification can lead to linear as well as macrocyclic products. The latter is formed when transesterification occurs via back biting reactions. These transesterification reactions will broaden the molecular-weight distribution.^{21,23,24,25} Perhaps, transesterification occurs less frequently during step-condensation polymerizations than chain-end polymerizations. More research will be needed to provide definitive answers that

explain the differences in PDI observed for lipase-catalyzed step-condensation and chain polymerizations.

GPC Monitoring of Chain Growth. The evolution of molecular weight distribution as a function of time was studied for Novozyme-435-catalyzed condensation of 12-hydroxydodecanoic acid in bulk at 90 °C. Figure 2a displays GPC traces of products for reaction times of 15 min, 1, 2, 4, and 24 h, as well as the chromatogram for a control reaction with a deactivated catalyst (90 °C, 48 h). The peak positions of oligomers marking their peak molecular weights (M_p) is designated by letters (Figure 2a). Areas under peaks were determined by cutting and weighing to approximate the relative formation of oligomers of differing M_p . Comparing these areas for 15 min and 4 h time points shows that the polymerization reaction is rapid. At 15 min and 4 h, the percent of products with M_p 0–380, 800–1300, 1860–2400, 2930, and >2930 was 14 vs 3, 26 vs 10, 17 vs 5, 6 vs 0, and 37 vs 82, respectively. By 24 h, the percent of chains with oligomers >2930 was 91%, indicating an uninterrupted increase in higher molecular-weight chains over time, with the concurrent depletion of oligomers with $M_p \leq 2930$.

The above results are consistent with the retention of a substantial fraction of the original enzymatic activity throughout the 48 h polymerization (see below). Observing the GPC trace for the control reaction verifies that chain growth occurs due to enzyme catalysis. The general pattern of chain build up with time is consistent with the results reported by Binns et al.^{4,11} for condensation polymerizations of unactivated diacids and diols. They described a solventless polycondensation between 1,4-butanediol and adipic acid catalyzed by immobilized CALB at 40 °C, showing that products formed after 4 h were primarily oligomers with DP_{avg} from 2 to 5. After the reaction was extended for 17 h at 60 °C in vacuo (100 mbar), the oligomer fraction was reduced to 10%. By focusing on the initial oligomerization sequence and identifying oligomeric reactants and products, they showed unambiguously that polyester chain formation occurred by a step-growth mechanism.

Effect of Water Removal. To study water content’s effect on lipase-catalyzed ω -hydroxyacid polymerizations, reactions were conducted identically except that a vacuum (10 mm of Hg) was or was not applied to remove water that evolved during the reactions. Figure 3 shows that water removal (or accumulation) can greatly change the outcome of polymerizations. Unexpectedly, by not removing water in vacuo, a remarkable increase in the extent of chain growth was observed at reaction times ≤ 4 h. For example, the DP_{avg} of poly(ω -hydroxydodecanoate) formed by 1 h was 5 with reduced pressure and 52 without (see Figure S3 in the Supporting Information), whereas by 4 h, the DP_{avg} was 25 with reduced pressure and 90 without. A comparison of Figures 2b and 4 shows the large difference in the evolution of various poly(ω -hydroxydodecanoate) molecular-weight fractions depending on whether water was actively removed from the reaction. By 4 h, for reactions with and without application of vacuum, the percent of products with M_p 0–380, 800–1300, 1860–2400, 2930, and >2930 was 3 vs 0, 10 vs 0, 5 vs 0, 0 vs 0, and 82 vs 100, respectively.

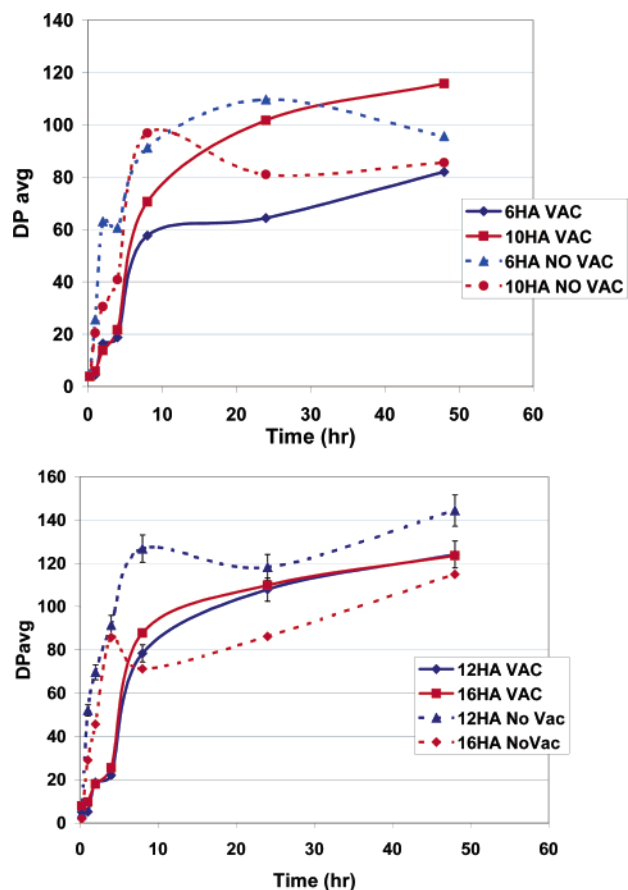


Figure 3. Effect of water removal on extent of chain growth with time for Novozyme-435-catalyzed condensation polymerization of ω -hydroxyacids (90 °C, in bulk, 10wt %/wt catalyst:monomer). The error bars are calculated from triplicate runs.

Because reactions were conducted at 90 °C in 20 mL flasks with 600 mg of monomer, water that evolved at ambient pressure during condensation reactions collected on the walls of the flask, largely outside the reaction mixture. Of the 8% water expected in the reaction mixture from condensation reactions, titration showed only 4% remained after 48 h (see the Experimental Section). Only 0.3% remained after 48 h with application of vacuum. By 48 h, the DP_{avg} of products formed with and without application of vacuum was similar. This slowing of reactions run without water removal after 4 or 8 h (see Figure 3) may be due to many factors such as the catalysis of hydrolytic reactions, partitioning of the substrate, inactivation of the enzyme, agglomeration of the enzyme particles, and back-biting reactions that give macrocyclic products. Furthermore, chain growth slows in bulk polymerizations due to diffusion constraints. Nevertheless, the results in Figures 2b, 3, and 4 demonstrate that important improvements in the rate of chain growth result by manipulating the content of water in the polymerization reactions.

The logic behind efficient water removal in condensation polymerizations is to shift the thermodynamic equilibrium, though this does not take into consideration that enzymes require certain water levels in their local environment to function efficiently. This explains why sub-optimal water content may provide insufficient flexibility for an enzyme to maintain its unique catalytically competent conformation.^{26,13} Indeed, the regulation of water activity in reactions

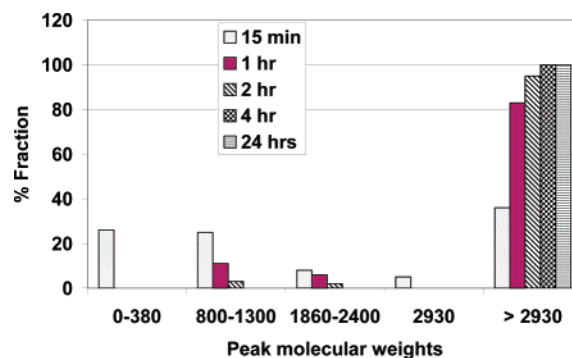


Figure 4. Percent by wt relative to the total sample of selected molecular species are found in the product as a function of reaction time. The values were determined by GPC traces of a Novozyme-435-catalyzed polymerization of 12-hydroxydodecanoic acid (90 °C, in bulk, 10 wt %/wt catalyst:monomer, without application of a vacuum).

has been used to optimize lipase-catalyzed transesterifications of low-molar-mass substrates. For example, Lee et al.²⁶ showed that the reaction yield for lipase-catalyzed esterification of *n*-capric acid with *n*-decyl alcohol was significantly improved with water-activity control. They observed that conversions obtained at 28 h with and without water-activity control were 70 and 96%, respectively. They found that the optimal condition was to maintain water activity at 0.55 (until 11 h) followed by 0 water activity. This approach attained a final conversion of 100%.²⁶ Unfortunately, the optimal water level differs with different reaction systems, so we cannot extrapolate from the work of Lee et al.²⁵ to the present system. However, we can conclude from the present work that, when a vacuum was applied for at least 4 h, the reactions' water content fell below what was needed to retain optimal enzyme activity (Figure 3).

Although no reports on the effect of water removal on the polymerization of hydroxyacids were found, the effect of water removal on the polyesterification between sebacic acid and 1,4-butanediol was published by Linko et al.⁶ They studied the effect of water removal over long reaction times (360 h) and concluded water removal was essential to obtain higher-molecular-weight products. Unfortunately, insufficient data points were taken at the initial stages of the polymerization (less than 8 h) to determine water's role in the early stages of the reaction. Previously, we reported that until 24 h (M_n 12 000) there was no significant effect of water removal on the condensation polymerization of adipic acid and 1,8-octanediol.²⁷ Beyond 24 h, water removal increased the molecular-weight buildup. This result illustrates how differing reaction systems may respond another way to water removal and have dissimilar optimal water levels. Currently, research is underway in our laboratory to study reaction systems that allow for the control of water levels. From these studies, we hope to determine how reaction water content can be manipulated to further improve the kinetics of lipase-catalyzed condensation polymerizations.

Effect of Catalyst Concentration. Experiments were performed using 12-hydroxydodecanoic acid as a model system at 90 °C in vacuo. Novozyme-435 concentrations studied were 10, 1, 0.50, and 0.10%-by-wt relative to monomer (Figure 5). Because Novozyme-435 is comprised

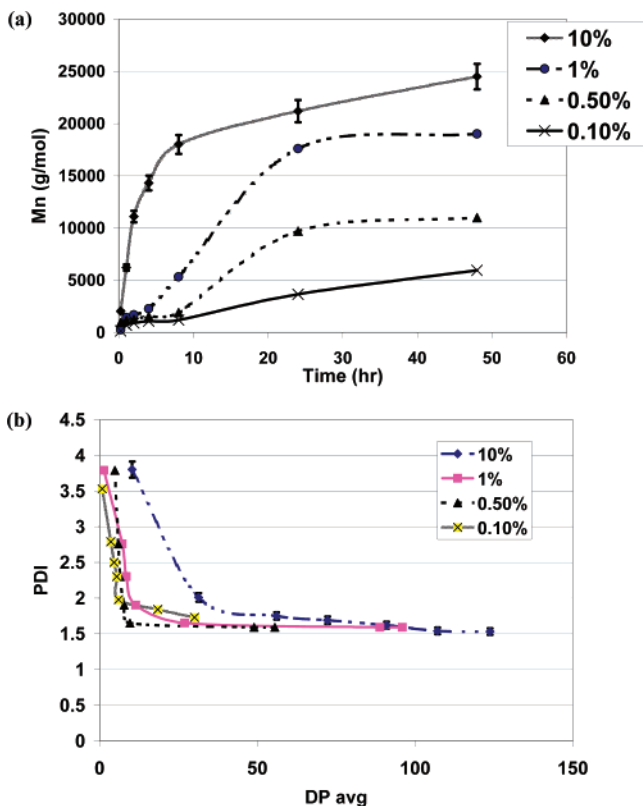


Figure 5. Effect of catalyst concentration for the polymerization of 12-hydroxydodecanoic acid (90 °C, in bulk, with application of vacuum) on (a) the extent of chain growth as a function of time and (b) the polydispersity index (PDI) of products formed. The error bars were calculated from triplicate runs.

of 10%-by-wt CALB on Lewatit, the corresponding %-protein in these reactions was 1, 0.1, 0.05, and 0.01%-by-wt, respectively. Figure 5a shows that, by 8 h, the M_n of products obtained at these catalyst concentrations were 18 000, 5050, 2000, and 1500, respectively. By 24 h, the M_n of products at these catalyst concentrations were 21 500, 18 000, 9800, and 4000, respectively. Thus, although polymerizations with 1% protein took place relatively faster than those with 0.1% protein, by 24 h, the products from these reactions with 1 and 0.1% protein had similar molecular weights. Figure 5b shows, within the range of catalyst concentrations between 0.5 and 10%, all of the polyesters formed had narrow dispersity ($PDI \leq 1.5$).

Catalyst Activity versus Reaction Time. Previously, we reported that Novozyme-435-catalyzed bulk polycondensations between diacids and diols at 70 °C for 48 h gave a recovered catalyst with approximately 78% of its original activity.¹⁴ For this paper, the polymerization temperature used was 90 °C. The activity of the recovered catalyst for bulk polycondensations of 12-hydroxydodecanoic acid at 90 °C was assayed by a GC method where the extent of propyl laurate formed in toluene was determined.¹⁴ Figure 6 shows that by 4, 24, and 48 h, the residual activity of the catalyst beads dropped to 93, 82, and 75%, respectively. Thus, even after a 48 h reaction at 90 °C, the catalyst retains almost 75% of its original activity, making reuse of the recovered catalyst for at least another polymerization cycle seem feasible. In contrast, Binns et al.⁴ reported that recovered Novozyme-435 beads had 35% of their original activity for

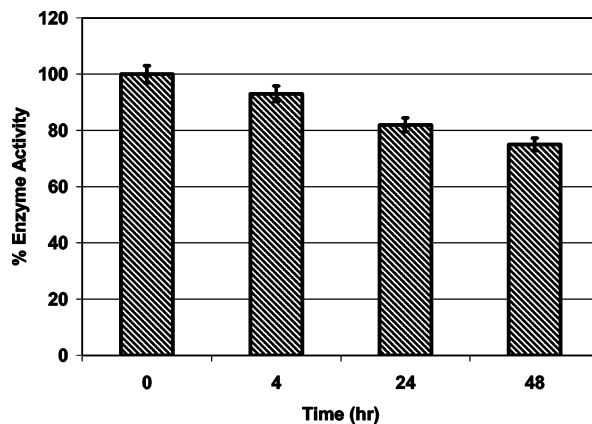


Figure 6. Retention of enzyme activity as a function of reaction time for the condensation polymerization of 12-hydroxydodecanoic (90 °C, in bulk, 10 wt %/wt catalyst:monomer). The error bars were calculated from triplicate runs.

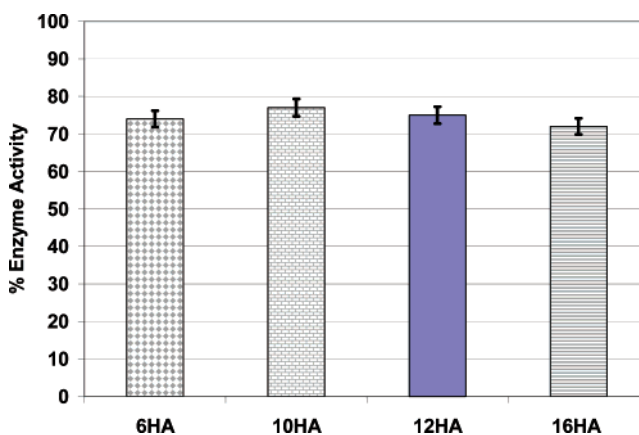


Figure 7. Effect of monomer substrates on retention of enzyme activity for the condensation polymerization of 12-hydroxydodecanoic acid (90 °C, in bulk, 48 h, 10 wt %/wt catalyst:monomer). The error bars were calculated from triplicate runs.

an in-bulk butanediol/adipic acid copolymerization conducted in vacuo for 17 h at 60 °C. Because details of how reactants were mixed were not described, it may be that larger shear applied to the catalyst particles resulted in larger extents of lost catalyst activity. The detailed factors that influence changes in enzyme activity as a function of reactor design and operation are currently under study in our laboratory.

The %-activity remaining after 48 h bulk polymerizations of the different hydroxyacids studied was found to be similar (70–80%; see Figure 7). So differences in the reaction rate of 6-hydroxyhexanoic acid and the longer chain hydroxyacids (see Figure 1a) are not attributable to dissimilarities in retained catalyst activity during the reactions.

Summary of Results

Polymerizations of linear aliphatic hydroxyacids were successfully performed in bulk, at 90 °C, using 10% w/w relative to monomer of Novozym 435 (macroporous beads that contain 10% w/w of lipase B from *Candida antarctica*). There was little difference in the time profile of DP_{avg} values for ω -hydroxyacids with chain lengths of C-16, C-12, and C-10. These polymerizations gave products with $DP_{avg} \sim 105$ and M_w/M_n of 1.5 by 24 h. However, the shorter chain

substrate 6-hydroxyhexanoic acid polymerized relatively slower, so that by 24 h, poly(6-hydroxyhexanoate) was formed with $DP_{\text{avg}} \sim 63$ and M_w/M_n 1.6. The apparent insensitivity to ω -hydroxyacid chain lengths between C-10 and C-16 was surprising given the general chain-length selectivity of lipases. Similar to Novozyme-435-catalyzed diacid/diol polycondensations, the M_w/M_n values tended toward 1.5 as the polymerizations progressed. This result is in contrast to M_w/M_n values of about 2.0 for Novozyme-435-catalyzed lactone polymerizations. Currently, the fundamental aspects of the polymerization mechanism that lead to these very different polydispersity values are not understood. Plausible explanations are that the molecular weight of lactone polymerizations is broadened by the slow initiation of chains and by a greater tendency (than in condensation polymerizations) to undergo transesterification reactions. Nevertheless, the narrow dispersity of polymers from Novozyme-435 condensation polymerizations can provide beneficial solid-state properties such as enhanced crystallization rates.

A remarkable increase in chain build up occurred when water was not removed from ω -hydroxyacid polycondensations. This observation is consistent with reports where, by controlling the water contents in reactions, improved kinetics of transesterification reactions were achieved for low-molar-mass compounds. This use of reaction water concentration to accelerate lipase-catalyzed polycondensations has not previously been reported. Further study of reaction water content using well-controlled systems is expected to result in important improvements for a wide range of lipase-catalyzed polycondensation reactions.

This paper also showed that a recovered catalyst from bulk polycondensations of ω -hydroxyacids at 90 °C for 48 h retains about 75% of its original activity. This illustrates the remarkable thermal stability of Novozyme-435.

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Supporting Information Available. Expanded views of Figures 1a and 3 (Figures S1 and S3, respectively). GPC traces versus time for condensation polymerization of 12-hydroxydodecanoic acid (Figure S2). Plot of the effect of water removal on molecular-weight distribution (polydispersity) with time for Novozyme-435 catalyzed—condensation polymerizations of linear aliphatic hydroxyacids (Figure S4). This material is available free of charge via the Internet at <http://pubs.acs.org>.

Note Added after ASAP Posting. This article was initially posted ASAP on 11/8/2003 without a Supporting Information paragraph. The correct version was posted on 12/23/2003.

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