Lipase-Catalyzed Ring-Opening Polymerizations of 4-Substituted ϵ-Caprolactones: Mechanistic Considerations

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Received March 9, 2005; Revised Manuscript Received May 3, 2005

ABSTRACT: Lipase-catalyzed ring-opening polymerizations of 4-substituted ϵ-caprolactones employing Novozym 435 as the biocatalyst demonstrate dramatic differences in polymerization rates and selectivity depending on the size of the substituent. Quantification of the reaction rates shows that the polymerization rate decreases by a factor of 2 upon the introduction of a Me substituent at the 4-position. Moreover, 4-EtCL polymerizes 5 times slower than 4-MeCl and 4-PrCL is even 70 times slower. The decrease in polymerization rate is accompanied by a strong decrease in enantioselectivity: while the *E*-ratio of 4-MeCL polymerization is 16.9, the *E*-ratios of 4-EtCL and 4-PrCL are 7.1 and 2.0, respectively. Interestingly, Novozym 435 displays S-selectivity for 4-MeCL and 4-EtCL in the polymerization reaction, but the enantioselectivity is changed to the (*R*)-enantiomer in the case of the 4-PrCL. The nature of these differences was investigated by hydrolyzing all monomers in water/diisopropyl ether mixtures employing Novozym 435 as the catalyst. In the hydrolysis reactions, the rates are only moderately affected upon increasing the substituent size, and the enantioselectivity is S in all cases, also for 4-PrCL. Again, a steady decrease of the *E*-ratio was observed upon increasing the substituent size, but this was less pronounced than in the polymerization reactions: the *E*-ratios were 17.6, 12.4, and 4.6, going from 4-MeCL to 4-PrCL. For 4-substituted ϵ-caprolactones, the results obtained are a clear indication that the chirality of the propagating alcohol chain end is important in the catalytic cycle and that—in contrast to unsubstituted lactones—the rate-determining step is not necessarily the formation of the acyl-enzyme intermediate but more likely the deacylation of the acyl-enzyme intermediate by the propagating alcohol chain end.

Introduction

Lipase-catalyzed ring-opening polymerizations (ROP) of lactones have become a valuable tool to prepare well-defined polyesters.[1] Increasingly popular is the lipase B of *Candida antarctica* (CALB) immobilized on an acrylic resin and commercially available as Novozym 435. This lipase was found to be an exceptionally stable and highly active catalyst in the ROP of lactones and carbonates.[2] Moreover, the intrinsic ability of CALB to perform reactions in an enantioselective way could be exploited to synthesize chiral polymers and block copolymers from racemic mixtures of substituted lactones via kinetic resolution ROP.[3−6]

In lipase-catalyzed ROP, it is generally accepted that the lactone activation proceeds via the formation of an acyl-enzyme intermediate by reaction of the serine residue with the lactone rendering the carbonyl prone to nucleophilic attack (see Scheme 1).[3,7−11] Initiation of the polymerization occurs by deacylation of the acyl-enzyme intermediate by an appropriate nucleophile such as water or an alcohol to produce the corresponding ω-hydroxycarboxylic acid/ester. Propagation, on the other hand, occurs by deacylation of the acyl-enzyme intermediate by the terminal hydroxyl group of the growing polymer chain to produce a one unit elongated polymer chain. Careful mechanistic investigations in lipase-catalyzed ROP of unsubstituted lactones revealed that these polymerizations follow Michaelis−Menten kinetics and that the formation of the acyl-enzyme intermediate is the rate-determining step.[3] The determination of the Michaelis−Menten constant, K_M, and V_{max} of lactones of varying ring sizes showed that K_M is relatively independent of the ring size while V_{max} increases with increasing ring size. This suggests that the binding abilities of lipases are independent of the lactone ring size and points to the formation of the acyl-enzyme intermediate as the key step in these polymerizations. These results were corroborated by several groups, and it appears that all lipases, including CALB, obey the rule that monomer activation is the rate-determining step for ROP.[8−11]

We have recently studied enantioselective ROP of substituted ϵ-caprolactones catalyzed by CALB and found that the rate of polymerization is dependent on the position of the substituent on the lactone ring. 5-Methyl-ϵ-caprolactone showed reasonable fast polymerization rates, 3-methyl-ϵ-caprolactone and 4-methyl-ϵ-caprolactone showed moderately fast polymerization rates, and 6-methyl-ϵ-caprolactone (6-MeCL) did not polymerize.[6a] In the latter case, (*S*)-6-MeCL is the faster reacting enantiomer, but the formation of an (*S*)-alcohol end group after ring-opening hampers propagation as a result of the stereospecificity of CALB for (*R*)-secondary alcohols.[12] The importance of the stereoconfiguration of the secondary alcohol on the propagation rate in 6-MeCL polymerizations raises the question of whether the polymerization rate of other substituted lactones is also increasingly influenced by the nature of the propagating (chiral) alcohol. Preliminary indications for this hypothesis were found when we compared previously published results on the polymerization of 4-methyl-ϵ-caprolactone and 4-ethyl-ϵ-caprolactone: both the rate and the enantioselectivity of the polymerization decreased upon increasing the substituent size.[5]

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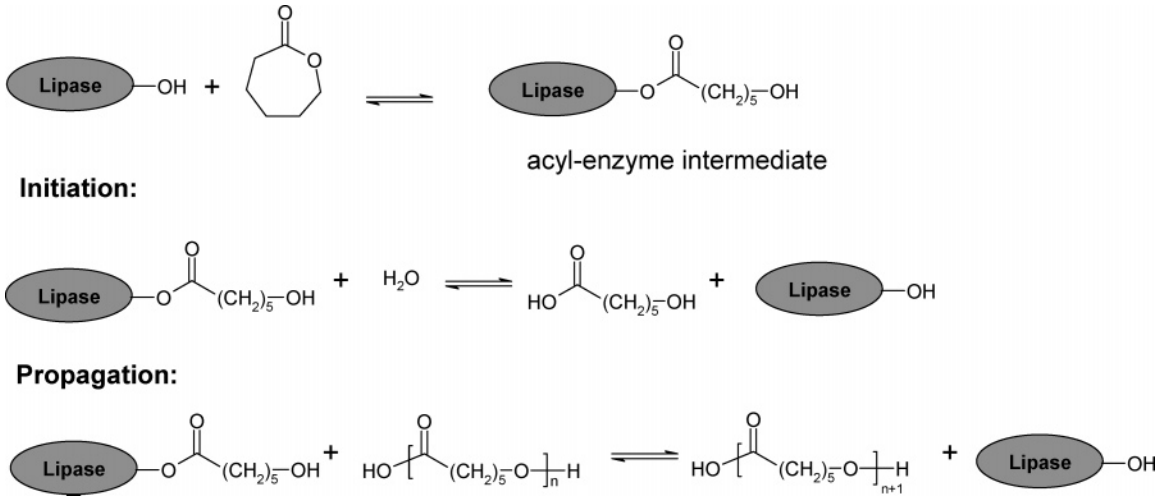
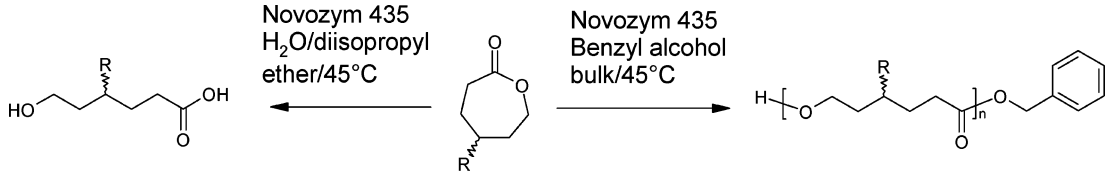
10.1021/ma050510j CCC: $30.25
Published on Web 06/01/2005

Scheme 1. Proposed Mechanism for Lipase-Catalyzed ROP of Lactones[7]



Scheme 2. Lipase-Catalyzed Hydrolysis and ROP of ϵCL and 4-Substituted ϵ-Caprolactones



εCL: R = H
4-MeCL: R = CH_3
4-EtCL: R = CH_2CH_3
4-PrCL: R = $CH_2CH_2CH_3$

Here we report on our detailed studies regarding the influence of the substituent size on the rate and enantioselectivity of CALB-catalyzed ring-opening polymerizations in the series 4-methyl-ϵ-caprolactone (4-MeCL), 4-ethyl-ϵ-caprolactone (4-EtCL), and 4-propyl-ϵ-caprolactone (4-PrCL) (Scheme 2). 4-PrCL is an especially interesting monomer for this study since the size of the substituent ($CH_2CH_2CH_3$) is comparable to the size of the leaving group (CH_2CH_2OH) upon ring-opening of the lactone. The polymerization of ϵ-caprolactone (ϵCL) was included as a reference. Furthermore, CALB-catalyzed hydrolysis reactions of this series of 4-substituted ϵ-caprolactones and ϵCL were performed to assess the importance of the nucleophile in these reactions (Scheme 2). The enantioselectivities as well as the rates of all reactions were carefully quantified. The results are used to validate our hypothesis that in kinetic resolution polymerizations of substituted ϵ-caprolactones both acylation and deacylation steps occur enantioselectively and that deacylation of the acyl-enzyme intermediate is the rate-determining step in the polymerization reaction.

Materials and Methods

Materials. All chemicals were purchased from Aldrich and used without further purification unless otherwise noted. Prior to use, benzyl alcohol and all ϵ-caprolactones were freshly distilled from CaH_2 under reduced pressure and stored over 4 Å molecular sieves. Novozym 435 was purchased from Novozymes A/S.

Analytical Methods. Reactions were followed by chiral gas chromatography (GC) with a Shimadzu 6C-17A GC equipped with an FID employing a Chrompack Chirasil-DEX CB (DF = 0.25) column. Injection and detection temperatures were set at 300 and 325 °C, respectively. Separations were done under isothermal conditions with the column temperature set at 125 °C for 4-MeCL, 130 °C for 4-EtCL, and 140 °C for 4-PrCL; in all cases baseline separation of the enantiomers was achieved. All samples were measured in duplo using a Shimadzu AOC-20i autosampler. The ee_m was calculated as follows: $ee_m = (R - S)/(R + S)$, where R and S represent the surfaces of the GC peaks of the (*R*)- and (*S*)-enantiomer, respectively. The enantiomeric ratio (*E*-ratio)[13] which in terms of conversion (c) and ee_m is expressed as $E = \ln[(1 - c)(1 - ee_m)]/\ln[(1 - c)(1 + ee_m)]$ was fitted using Origin 6.0 employing the nonlinear curve fit option and rewriting the formula to $c = 1 - [(1 - ee_m)/(1 + ee_m)^E]^{(1/E-1)}$. For the hydrolysis reactions, the lactone conversions were determined by chiral GC using the internal standard method with di-*tert*-butylbenzene as the internal standard. For the polymerization of the 4-substituted lactones in bulk, the conversion, c, was determined from ^{1}H NMR spectra by comparing the intensity of the C*H*$_2$C=O peak at 2.65 ppm ($I_\delta = 2.25$) of the monomer to the intensity of the C*H*$_2$C=O peak at 2.30 ppm of the polymer ($I_\delta = 2.30$) so $c = (I_\delta = 2.30)/[(I_\delta = 2.30) + (I_\delta = 2.65)]$. The initial rate constants (k_i) of the faster reacting enantiomers were derived from the slope of the $\ln(1 - c)$ vs time plot assuming first-order kinetics. ^{1}H and ^{13}C NMR spectra were taken with a Varian Mercury 400 or Gemini 300 spectrometer (400 or 300 MHz, respectively) in $CDCl_3$ with the delay time (d_1) set at 10 s. Gel permeation chromatography (GPC) was carried out on a Waters 712 WISP HPLC system with a Waters 410 differential refractometer detector and a PL gel guard precolumn (5 mm, 50 × 7.5 mm) followed by two PL gel mixed-C columns (10 mm, 300 × 7.5 mm, Polymer Laboratories), using THF as the eluent. All molecular weights were relative to polystyrene standards. The water content of diisopropyl ether was determined using a Mettler-Toledo DL 39 Karl Fischer coulometric titrator with CombiCoulomat fritless (Merck) as the electrolyte.

Monomer Synthesis and Characterization. Baeyer–Villiger oxidations were performed as described previously.[6a] The peaks in the GC traces were assigned by comparison with literature data.[5,14] 4-Methyl-ϵ-caprolactone: yield = 85%; bp

= 58 °C/0.04 Torr. ^{1}H NMR ($CDCl_3$): δ 4.25 (m, 2H, CH_2OC=O); 2.65 (m, 2H, CH_2C=O); 2.00−1.15 (5H, CH and 2 × CH_2); 1.02 (d, 3H, CH_3).^{13}C NMR ($CDCl_3$): δ 176.0 (C=O); 68.0; 37.2; 35.1; 33.1; 30.7; 22.1. GC retention time (*S*)-4-MeCL = 15.3 min, (*R*)-4-MeCL = 15.7 min. 4-Ethyl-ϵ-caprolactone: yield = 76%; bp = 74 °C/0.05 Torr. ^{1}H NMR ($CDCl_3$): δ 4.20 (m, 2H, CHOC=O); 2.60 (m, 2H, CH_2C=O); 1.90−1.20 (7H, CH and 3 × CH_2); 0.95 (t, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 175.9 (C=O); 68.8; 41.6; 34.8; 32.9; 28.9; 28.3; 11.1. GC retention time (*S*)-4-EtCL = 21.4 min; (*R*)-*4*-EtCL = 22.1 min. 4-Propyl-ϵ-caprolactone*:* yield = 80%; bp = 90 °C/0.05 Torr. ^{1}H NMR ($CDCl_3$): δ 4.20 (m, 2H, CH_2OC=O); 2.60 (m, 2H, CH_2C=O); 1.90−1.20 (9H, CH and 4 × CH_2); 0.95 (t, 3H, CH_3). ^{13}CMR ($CDCl_3$): 176.0 (C=O); 68.0; 39.7; 38.5; 35.2; 33.0; 28.7; 19.7; 13.9. GC retention time (*S*)-*4*−PrCL = 20.3 min; (*R*)-4-MeCL = 21.0 min.

General Procedure for the Hydrolysis of ϵ-Caprolacton and 4-Substituted ϵ-Caprolactones with Novozym 435. The appropriate lactone (7.85 mmol) and water (39.22 mmol) were mixed in diisopropyl ether (5.0 g) and di-*tert*-butylbenzene (303 mg, 1.59 mmol) and stirred at 45 °C. Di-*tert*-butylbenzene was added as internal standard to calculate conversions from the GC traces. Then, Novozym 435 (600 mg) was added. Samples (0.1 mL) were taken at regular time intervals, filtered over Na_2SO_4, and analyzed with GC. At the end of the reaction, the enzyme was removed by filtration, and the filtrate was evaporated in vacuo.

General Procedure for the Polymerization of ϵ-Caprolacton and 4-Substituted ϵ-Caprolactones with Novozym 435. Novozym 435 (200 mg) was weighted in an oven dried Schlenck tube. Then, the tube was provided with a stirring bar and rubber septum and put overnight in a vacuum oven at 50 °C. Some P_2O_5 was put (separately) in the oven to ensure a dry atmosphere. The vacuum was released by backfilling the oven with nitrogen, and some 4 Å molecular sieves were added to the tube. The reaction tube was then filled with argon, and the stock solution of lactone/benzyl alcohol in a 50/1 ratio (2.03 g) was added through the septum (the ratio of lactone to enzyme is kept constant at 10% w/w). The reaction was stirred at 45 °C. Samples (0.1 mL) were taken at regular time intervals and analyzed with ^{1}H NMR, GC, and GPC. In the case of 4-PrCL, the polymer was precipitated in heptane and obtained as a clear oil. The filtrate was collected and evaporated in vacuo. The remaining oil was distilled and afforded enantioenriched 4-PrCL: $[\alpha]_D = -4.3$ (neat) (lit.:[15] $[\alpha]_D = -38$ for pure (*S*)-4-PrCL).

Results and Discussion

Ring-Opening Polymerization of 4-Substituted ϵ-Caprolactones with Novozym 435. To assess the influence of the size of substituents on the enantioselectivity and polymerization rates of CALB-catalyzed ROP of substituted ϵ-caprolactones, we performed ROP of ϵCL, 4-MeCL, 4-EtCL, and 4-PrCL in bulk at 45 °C. In all cases, benzyl alcohol was employed as the initiator in a 1/50 ratio of initiator to monomer to regulate the molecular weight of the polymers. For all polymerizations, the overall lactone conversions were plotted as a function of time (see Figure 1a), and the first-order rate constants were determined for the faster reacting enantiomer. The results are summarized in Table 1 and compared with those of ϵCL.

The chiral GC traces show that Novozym 435 is *S*-selective for 4-MeCL and 4-EtCL. The *S*-selectivity for 4-MeCL and 4-EtCL is analogous to the results previously found by Bisht et al.[5] Surprisingly, Novozym 435 is *R*-selective for 4-PrCL, which was confirmed by isolating nonreacted monomer after the polymerization. Purification of the isolated 4-PrCL showed a negative optical rotation, which is indicative for an enantiomeric excess of (*S*)-4-PrCL.[15] The enrichment of the (*S*)-enantiomer in the unreacted monomer indicates that the (*R*)-enantiomer is the faster reacting enantiomer.

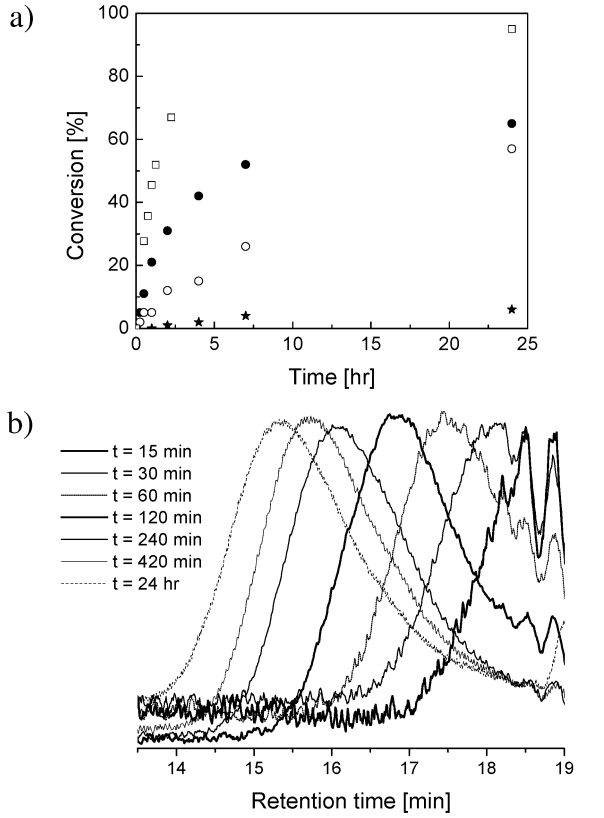


Figure 1. (a) Overall conversion of ϵ-CL (□), 4-MeCL (●), 4-EtCL (○), and 4-PrCL (★) as a function of time in a Novozym 435-catalyzed ROP at 45 °C in bulk. (b) Molecular weight development during the Novozym 435-catalyzed polymerization of 4-MeCL conducted at 45 °C in bulk with a benzyl alcohol/monomer molar ratio of 1/50.

The time−conversion plots (Figure 1a) show a significant decrease of the polymerization rates upon the introduction of a substituent at the 4-position. ϵCL is consumed fastest, and the overall conversions after 24 h of ϵCL, 4-MeCL, 4-EtCL, and 4-PrCL are >95%, 65%, 57%, and 6%, respectively. The significant differences in polymerization rates are further substantiated by comparing the initial rate constants of all faster reacting enantiomers with the initial rate constant of the ϵCL polymerization: k_i of ϵCL is 2, 10, and 137 times higher than the k_i of (*S*)-4-MeCL, (*S*)-4-EtCL, and (*R*)-4-PrCL, respectively (Table 1).

In addition, molecular weights and molecular weight distributions were measured from samples taken during the polymerization reaction (Table 1). As an example, the GPC traces of samples withdrawn during the polymerization of 4-MeCL are depicted in Figure 1b. As expected, the molecular weight gradually increases over time in all cases. The final molecular weight obtained for 4-PrCL, 1700 g/mol, is lower than for 4-MeCL and 4-EtCL—6200 and 4100 g/mol, respectively—which is in accordance with the overall conversions reached. The molecular weights attained are in good agreement with the calculated molecular weights of ∼4300, ∼4100, and ∼1700 g/mol for P(4-MeCL), P(4-EtCL), and P(4-PrCL), respectively, taking into consideration that the molecular weights of the substituted polycaprolactones are determined relative to polystyrene. In all cases, the polydispersities approach 2.

Table 1. Results of the Lipase-Catalyzed ROP of ϵCL and 4-Substituted ϵ-Caprolactones at 45 °C

lactone	faster reacting enantiomer	total conv [%] (time [h])	k_i[a] [h^{-1}]	$M_{n,calc}$[b] [g/mol]	M_n[c] [g/mol]	PD[c]	*E*-ratio
ϵCL	n.a.[f]	>95 (24)	0.684	3927[d]	4100[e]	2.2[e]	n.a.
4-MeCL	S	65 (24)	0.350	4268	6200	2.0	16.9 ± 2.3
4-EtCL	S	57 (24)	0.070	4155	4100	2.2	7.1 ± 0.6
4-PrCL	R	21 (72)	0.005	1746	1700	1.4	2.0 ± 0.1

[a] Faster reacting enantiomer. [b] $M_{n,calc} = M_{BA} + 50 \times conv \times M_{lactone}$, where M_{BA} and $M_{lactone}$ are the molecular weights of benzyl alcohol and the appropriate lactone, respectively. [c] Determined by GPC, relative to polystyrene standards. [d] Calculated at a conversion of 67%. [e] Determined at a conversion of 67%. [f] n.a. = not applicable.

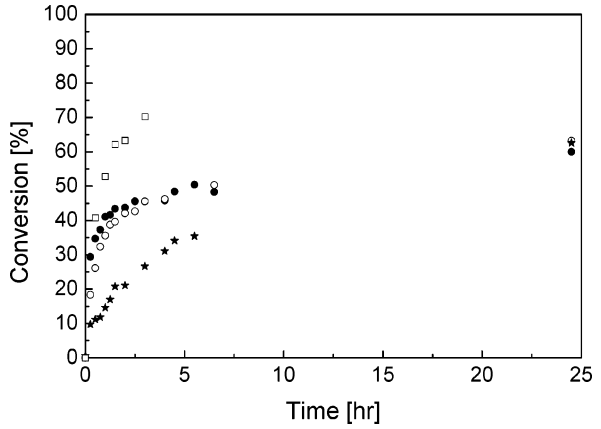


Figure 2. Overall conversion of ϵ-CL (□), 4-MeCL (●), 4-EtCL (○), and 4-PrCL (★) as a function of time in a Novozym 435-catalyzed hydrolysis at 45 °C.

From the samples taken during the polymerizations of 4-MeCL, 4-EtCL, and 4-PrCL, the enantiomeric excess of the nonreacted monomer (ee_m) was determined with chiral GC as a function of conversion. From these plots, the enantiomeric ratios (*E*-ratio) could be determined by standard fitting procedures (see Table 1).[13] The *E*-ratios decrease fast from 16.9 to 7.1 and 2.0 upon increasing the substituent size from methyl to propyl. The low *E*-ratio of 2.0 found for the polymerization of 4-PrCL implies that the selectivity of CALB for this monomer is very low.

Hydrolysis of 4-Substituted ϵ-Caprolactones Catalyzed by Novozym 435. To get a better insight into the selectivity and kinetics of CALB upon using 4-substituted ϵ-caprolactones, lipase-catalyzed hydrolysis reactions were performed. In this case, only the acylation step (formation of the acyl-enzyme intermediate) is of importance, and this will provide us with information on the relevance of the deacylation step with regard to selectivity and rates in the polymerization reactions.

We selected diisopropyl ether as the solvent of choice and an excess of water as the nucleophile. This gives rise to a two-phase system since the solubility of water in diisopropyl ether at 45 °C is only about 6 mg/g. Given that a heterogeneous catalyst was used, the reactions were conducted in a three-phase system. To assess the reproducibility of the hydrolysis reactions conducted in a three-phase system, all reactions were performed in duplicate or triplicate. In all cases, the overall lactone conversions were plotted as a function of time (Figure 2), and the initial rate constants, k_i, of the faster reacting enantiomers were determined from the time−conversion plots. The results are summarized in Table 2.

The results of the kinetic plots of the reactions in duplicate and triplicate were practically identical as evidenced by the small standard deviations (Table 2). This indicates that—despite the use of a three-phase system—the reactions were fully reproducible. Interestingly, CALB shows *S*-selectivity in the hydrolysis reactions of all three substituted ϵ-caprolactones.

Table 2. Initial Rate Constants (k_i) and Selectivity (*E*-Ratio) for the Novozym 435-Catalyzed Hydrolysis of ϵCL and 4-Substituted ϵ-Caprolactones

lactone	faster reaction enantiomer	total conv [%] (time [h])	k_i [h^{-1}][a] (S.D. [h^{-1}])[e]	*E*-ratio[d]
ϵCL	n.a.[e]	70 (3)	0.597 (±0.110)[b]	n.a.
4-MeCL	S	60 (24)	1.376 (±0.092)[c]	17.6 ± 5.6
4-EtCL	S	63 (24)	1.242 (±0.090)[b]	12.4 ± 1
4-PrCL	S	63 (24)	0.234 (±0.024)[c]	4.6 ± 0.3

[a] Faster reacting enantiomer. [b] Average of two different experiments. [c] Average of three different experiments. [d] Fitted for one representative set of experiments [e] n.a. = not applicable; S.D. = standard deviation.

The time−conversion curves of the lactone hydrolysis reactions (see Figure 2) show rather similar rates of hydrolysis in all cases. After 24 h, the conversion levels off at a value of about 60−70%. Comparison of the initial rate constants reveals that the k_i of (*S*)-4-MeCL and (*S*)-4-EtCL are even higher than that of ϵCL. (*S*)-4-PrCL hydrolyses only about 3 times slower than ϵCL (Table 2).

From one set of hydrolysis reactions of 4-MeCL, 4-EtCL, and 4-PrCL conducted in parallel, the ee_m vs conversion was plotted from which the *E*-ratio could be determined by standard fitting procedures.[13] Table 2 shows that the *E*-ratios for 4-MeCL and 4-EtCL are rather similar (17.6 and 12.4, respectively) while the *E*-ratio of 4-PrCL is lower (4.6).

Discussion. CALB is extensively studied in kinetic resolutions of racemic secondary alcohols for which it displays extraordinary high enantioselectivities.[16] X-ray crystallographic studies indicated that the active site pocket is composed of two channels: one hosting the acyl moiety and one hosting the alcohol moiety of the substrate.[12] The high enantioselectivity of CALB in kinetic resolutions of secondary alcohols is explained by the presence of a stereoselectivity pocket in the alcohol-hosting channel. The acyl-hosting channel is less confined, and usually, a lower enantioselectivity is found for kinetic resolutions of esters, acids, and carbonates.[14,17] Similar to other serine hydrolases, a serine−histidine−aspartate catalytic triad is responsible for the catalytic action. In CALB, the catalytic triad consists of Ser-105, His-224, and Asp-187. The action of CALB relies on a two-step mechanism with an acylation step and a deacylation step separated by a covalent acyl-enzyme intermediate (see Scheme 3).[16] The first step is the binding of an acyl donor to Ser-105. This binding affords the first transition state, which is stabilized by hydrogen bonds from His-224 and the oxyanion hole. After an alcohol group leaves the complex, the acyl-enzyme intermediate remains (acylation of the enzyme). When an alcohol attacks, it forms the second transition state that is stabilized in the same way as the first one.

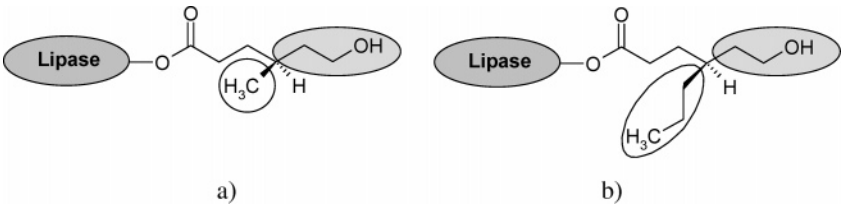
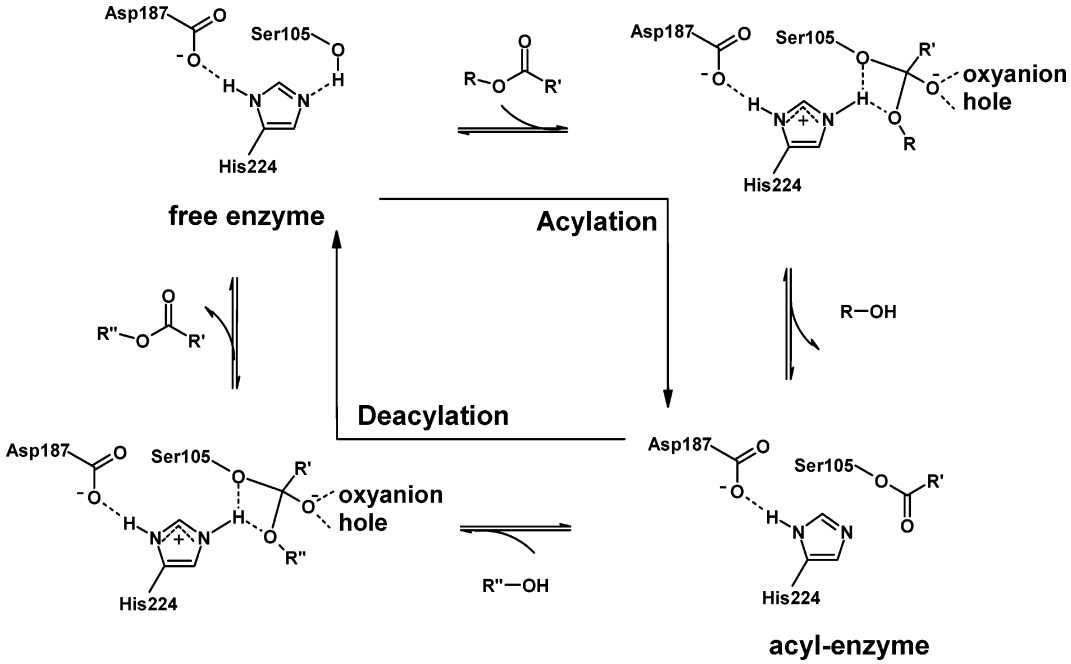


Figure 3. Schematic representation of the acyl-enzyme intermediates of (a) 4-MeCL and (b) 4-PrCL. Note the size difference between the methyl and propyl substituent in (a) and (b), respectively.

Scheme 3. Reaction Mechanism of CALB[16]



The product leaves the active site and the free enzyme remains (deacylation of the enzyme).

In kinetic resolution polymerizations of substituted ϵ-caprolactones both acylation and deacylation steps may occur enantioselectively (see Scheme 3). Enantiodiscrimination occurs in the formation of the acyl-enzyme intermediate but may also occur in the deacylation step since the alcohol formed after ring-opening—that serves as the propagating chain end—is chiral as well. This is nicely illustrated by the reluctance to polymerize of 6-MeCL, as discussed above.[6a]

We have made the following observations in the kinetic resolution polymerizations of 4-substituted ϵ-caprolactones: (1) the polymerization rate decreases upon the introduction of a substituent at the 4-position, (2) the polymerization rate further decreases upon increasing the substituent size from Me to Pr, (3) the enantioselectivity changes to the (*R*)-enantiomer in the case of the 4-PrCL, and (4) the selectivity decreases rapidly upon increasing the substituent size. Moreover, in the lipase-catalyzed hydrolysis reactions (1) the (*S*)-enantiomers of 4-MeCL and 4-EtCL hydrolyze faster than ϵCL, (2) the rates of hydrolysis are only moderately affected upon increasing the substituent size, (3) the enantioselectivity is *S* also for 4-PrCL, and (4) the selectivity decreases upon increasing the substituent size but not as significant as in the polymerization reactions.

These results suggest that deacylation of the enzyme must be rate determining in the polymerization of substituted ϵ-caprolactones while the rate-determining step in hydrolysis most likely is the formation of the acyl-enzyme intermediate. The latter makes sense since water is a very small nucleophile while a propagating alcohol chain end is not. The drop in selectivity of the hydrolysis reactions can be rationalized by the fact that the chirality in the acyl-enzyme intermediate seemingly decreases upon increasing the substituent size as tentatively explained in Figure 3. The differences in energy of the transition states in the case of (*R*)- and (*S*)-4-MeCL are expected to be more pronounced due to the large size difference between the Me substituent and the CH_2CH_2OH chain end. This is in contrast to the similar sizes of the $CH_2CH_2CH_3$ substituent and the CH_2CH_2OH chain end in the case of 4-PrCL. The more pronounced drop in selectivity in the case of the polymerization reaction can again be rationalized by taking the deacylation step into consideration: the propagating chain end is chiral as well, and hence it is logical to assume that one enantiomer fits better in the alcohol channel of the CALB active site than the other enantiomer. The enantiopreference of CALB for acyl donor and alcohol may be opposite as shown for the lack of polymerization in the case of 6-MeCL, and hence the overall selectivity and polymerization rate decrease even more. This may explain the change in selectivity in the case of 4-PrCL: both the monomer and the attacking alcohol chain end have to fit in the active site, and apparently in this case this results in a slight bias for the (*R*)-configuration in the monomer.

Unfortunately, there is, to our knowledge, no detailed data available on the enantioselectivity of acylating γ-substituted alcohols with CALB. However, the fact that prochiral 1,3-*meso*-diols and racemic 1,*n*-diols can

be resolved with other lipases may indicate that chiral centers remote from the nucleophilic site do influence enantioselectivity and rates in lipase-catalyzed polymerization reactions.[18]

Conclusions

There are obvious differences in rates and selectivity in lipase-catalyzed hydrolysis and polymerization reactions of 4-substituted ϵ-caprolactones. While the rate of hydrolysis is only slightly affected by the substituent size—a factor of 2 upon increasing the substituent size from Me to Pr—the rate of polymerization is decreased by a factor of 70. The change in selectivity from *S* to *R* going from 4-MeCL and 4-EtCL to 4-PrCL was unexpected, especially since the selectivity in the hydrolysis reactions was *S* for all substituted lactones. In addition, the enantioselectivity is more strongly influenced by the substituent size in the polymerization reactions compared to the hydrolysis reactions. For 4-substituted ϵ-caprolactones, the results presented above are a clear indication that the chirality of the propagating alcohol chain end is important in the catalytic cycle and that—in contrast to unsubstituted lactones—the rate-determining step is not necessarily the formation of the acyl-enzyme intermediate but more likely the deacylation of the acyl-enzyme intermediate by the propagating alcohol chain end.

Acknowledgment. The authors thank NRSC-C for financial support and Dr. J. A. J. M. Vekemans for stimulating discussions.

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MA050510J