Investigation of Lipase-Catalyzed Ring-Opening Polymerizations of Lactones with Various Ring Sizes: Kinetic Evaluation

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ABSTRACT: Lipase-catalyzed ring-opening polymerizations of lactones with various ring sizes (6- to 13- and the 16-membered ring) employing Novozym 435 demonstrate fascinating differences in their polymerization rates. These differences cannot be related to variations in physical properties such as the dipole moment of the lactones only. For example, 10-decanolactone, 11-undecanolactone, and 12-dodecanolactone show dipole moments of around 1.9 D, but the initial rate constant of their Novozym 435-catalyzed polymerization was found to be 0.10, 0.38, and 4.91 h^{-1}, respectively. The Michaelis−Menten constants K_M and V_{max} were measured for all lactones, and this revealed that the K_M was more or less independent of the ring size, suggesting similar affinities of the lipase for all lactones, while no obvious trend could be discerned for V_{max}. However, conformational strain and transannular interactions present in medium ring lactones (ring size 8−12) affect the reactivity of these lactones in lipase catalysis in a similar way as was previously described for the alkaline hydrolysis of lactones, rationalizing the low reactivity of the 10−12-membered rings and the high reactivity of the 8-membered ring. To our surprise, there is a large discrepancy in lipase-catalyzed ring-opening polymerizations compared to the alkaline hydrolysis reactions with respect to the relative reactivity of lactones possessing a cisoid conformation of the ester bond: while the ratio in rates between δ-valerolactone and 12-dodecanolactone in a alkaline hydrolysis is 9400, the ratio in rates using Novozym 435-catalyzed polymerization is only 3. In fact, in the latter case lactones possessing a cisoid conformation are less reactive than large ring lactones possessing a transoid conformation. This mechanistic study also furnished polyesters with an increasing number of methylenes in their repeat unit. The polyesters can be readily prepared in reasonable molecular weights (>10 000 g/mol). Preliminary thermal characterization of the polyesters shows, as expected, that the melting temperature and the melting enthalpy increase with an increasing number of methylenes in the repeat unit.

Introduction

Ring-opening polymerization (ROP) methods to convert lactones into well-defined, high molecular weight polyesters have been thoroughly investigated over the past 40 years and are nowadays well-established in polymer chemistry.[1] The resulting polyesters are applied as biodegradable and biocompatible materials in many applications.[2] ROP by organometallic "coordination−insertion" catalysts is commonly used since this gives rise to polymers with predictable molecular weights and low polydispersities.[3] However, to be suitable as a biomaterial, high-purity polyesters are required. Controlled ROP techniques based on organocatalysts have been developed which are an important tool to obtain metal-free polymers.[4] Moreover, nonmetal catalysts from natural sources like lipases are increasingly employed as biocatalysts for the preparation of well-defined polyesters.[5] The enzymatic ring-opening polymerization (eROP) of lactones with commercially available Novozym 435—an immobilized *Candida antarctica* Lipase B—is an easy and versatile method to prepare polyesters with well-defined end groups and controlled molecular weight.

Investigations on ROP of cyclic esters have focused primarily on strained cyclic (di)esters such as ϵ-caprolactone, lactide, and glycolide. A systematic study on the polymerizability of lactones of increasing ring size with $Zn(Oct)_2$ as the catalyst clearly showed a strong decrease in polymerizability of the lactones with increasing ring size.[6] In fact, long reaction times are required, and only low molecular weight polymers are obtained when macrolides such as ω-pentadecanolactone are polymerized with $Zn(Oct)_2$. At present, only yttrium isopropoxide displays fast and controlled polymerization of ω-pentadecanolactone.[7] With lipases, on the other hand, the polymerizability of lactones increases with increasing ring size when using the lipase of *Pseudomonas fluorescens* (Lipase PF).[6,8] Interestingly, also Novozym 435 polymerizes ω-pentadecanolactone faster than ϵ-caprolactone.[9] An explanation for the differences in behavior between lipases and organometallic catalysts is that chemical ROP strongly depends on the strain in the lactone ring. As a result, small rings such as δ-valerolactone and ϵ-caprolactone polymerize fast but larger rings such as ω-pentadecanolactone hardly polymerize. In lipases, factors other than ring strain govern the polymerizability, but it is not well-understood or generally accepted what these factors are. Although large ring lactones such as the ω-pentadecanolactone and the ω-hexadecanolactone resemble more the natural substrate of lipases, i.e., fatty acids, Michaelis−Menten studies on different lactones with Lipase PF as the catalyst showed similar affinities (K_M) for all lactones investigated but large differences in V_{max}.[8]

At present, no systematic study on the polymerizability of lactones of different ring size with Novozym 435 has been undertaken. Moreover, the factors governing lipase activity in lactone polymerization reactions have not yet been elucidated. Here, we present our studies on eROP of lactones of different ring size with Novozym 435 as the catalyst. We have measured the initial rate constants of the polymerization of lactones with rings sizes ranging form 6 to 13: δ-valerolactone (VL),

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ϵ-caprolactone (CL), 7-heptanolactone (HL), 8-octanolactone (OL), 9-nonanolactone (NL), 10-decanolactone (DL), 11-undecanolactone (UDL), and 12-dodecanolactone (DDL) and also the 16-membered ring, 15-pentadecanolactone (PDL). Moreover, we studied the Michaelis−Menten kinetics of all lactones in Novozym 435-catalyzed ring-opening polymerization reactions. Finally, we also present here our preliminary studies on the thermal properties of all polyesters obtained during the course of this study.

Experimental Section

Materials. Cyclic ketones (cycloheptanone, cyclooctanone, cyclodecanone, and cyclododecanone) and lactones (δ-valerolactone, ϵ-caprolactone, 11-undecanolactone, and 15-pentadecanolactone) were purchased from Aldrich and used without further purification unless stated otherwise. Toluene was freshly distilled from sodium prior to use. Novozym 435 was obtained from Novozymes A/S. All other reagents employed in this work were used as received.

Analytical Methods. All reactions were followed by 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 300 °C, respectively. The internal standard method, taking 1,3,5-tri-*tert*-butylbenzene as the internal standard, was used to determine the lactone conversion; all samples were measured using a Shimadzu AOC-20i autosampler. The initial rate constants (k_i) were derived from the slope of the ln(1 − conversion) vs time plot assuming first-order kinetics. For the analysis of the polymerization reactions in terms of Michaelis−Menten kinetics, polymerizations of lactones were conducted at different substrate concentrations with a fixed enzyme concentration. The initial rate of the lactone consumption (V_i) was calculated from the initial rate constants (see above) for each lactone concentration. Since we can assume that the concentration of nucleophile (alcohol initiator, water or alcohol chain end) remains constant during the polymerization, we can treat the polymerization reaction with pseudo one-substrate kinetics in which the lactone concentration decreases over time. We assume furthermore that in the initial stage of the polymerization the lactone (present in large excess) is the preferred substrate for CALB. Then, we can apply the well-known Michaelis−Menten equation. The initial rate, V_i, was plotted vs the lactone concentration according to the Hanes−Woolf plot [lactone]/V_i = (K_M/V_{max}) + ([lactone]/V_{max}), and from the slope of the linear fit and the intersection with the *y*-axis the Michaelis−Menten constant K_M and the maximal rate of polymerization, V_{max}, can be calculated.[8,10] To calculate the turnover number, k_{cat}, of Novozym 435, we assumed an active protein content of 2% w/w of the immobilized preparation as reported previously and a molecular weight of 33 000 g/mol for CALB.[11] ^{1}H and ^{13}C NMR spectra were taken with a Varian Mercury 400 or Varian Innova 500 spectrometer (400 or 500 MHz, respectively) in $CDCl_3$ with the delay time (d_1) set at 10 s. Chemical shifts are reported in ppm relative to tetramethylsilane. GC-MS spectra were taken with a Shimadzu GC-17A employing a Zebron-ZB-5 column (DF = 0.25 mm). Injector and detector temperatures were set at 300 °C. DSC spectra were measured with a Perkin-Elmer Pyris 1 DSC with heating and cooling rates of 20 K/min. The melting temperatures, T_m, and transition enthalpies, ΔH, were determined from the second heating run. Size exclusion chromatography (SEC) on poly(VL) and poly(CL) 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. High-temperature SEC measurements were done using *o*-dichlorobenzene (ODCB) as eluent at 80 °C on a PL-SEC 120 high-temperature chromatograph. A mixed-D column was used in combination with a flow rate of 1 mL/min and RI detector. Conventional RI calibration was performed using polystyrene standards for molecular weight determination. Before SEC analysis, all samples were heated, allowed to cool to room temperature, and filtered over a 5 μm PTFE filter. All molecular weights were relative to polystyrene standards.

Synthesis of Cyclononanone. Cyclononanone was synthesized via ring expansion of cycloheptanone according to a literature procedure[12] and was obtained as a colorless liquid in an overall yield of 17% (bp = 50 °C at 0.42 mbar). ^{1}H NMR ($CDCl_3$): δ 2.42 (m, 4H, CH_2C═O), 1.85 (m, 4H, CH_2CH_2C═O), 1.60 (m, 4H, CH_2), 1.40 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$): δ 218.4 (C═O), 43.6, 30.4, 26.9, 25.0, 24.3.

General Procedure for the Baeyer−Villiger Oxidations of Cyclic Ketones. The appropriate cyclic ketone (223 mmol) and *m*-chloroperbenzoic acid (275 mmol) were mixed in CH_2Cl_2 (250 mL). The suspension was heated under reflux until ^{1}H NMR showed complete consumption of the cyclic ketone. The reaction mixture was cooled in an ice bath, and the solids were filtered over Celite and washed with CH_2Cl_2 (2 × 50 mL). The filtrate was washed with 10% $Na_2S_2O_3$ solution (2 × 200 mL), saturated Na_2CO_3 solution (2 × 200 mL), and saturated NaCl solution (1 × 200 mL). The organic layer was dried with $MgSO_4$, filtered, and evaporated in vacuo. The resulting liquid was distilled over CaH_2 to afford the lactone in yields of around 70%. The purity of the lactone was determined using GC-MS, and all lactones were characterized with ^{1}H and ^{13}C NMR. *7-Heptanolactone:* Because of a competing hydrolysis reaction, the reaction was stopped after 3 days. After distillation, the purity of the lactone was 91%; bp = 41−43 °C at 1.4 mbar. ^{1}H NMR ($CDCl_3$): δ 4.28 (t, 2H, CH_2O), 2.48 (t, 2H, CH_2C═O), 1.75 (4H, m, CH_2), 1.52 (4H, m, CH_2). ^{13}C NMR ($CDCl_3$): δ 176.4, 67.6, 31.0, 30.5, 28.0, 25.4, 23.5. *8-Octanolactone:* The reaction was complete after 13 days; bp = 55−58 °C at 7.5 mbar. ^{1}H NMR ($CDCl_3$): δ 4.25 (t, 2H, CH_2O), 2.25 (t, 2H, CH_2C═O), 1.70 (6H, m, CH_2), 1.40 (4H, m, CH_2) ^{13}C NMR ($CDCl_3$): δ 175.4, 64.3, 35.4, 29.3, 27.6, 24.9, 23.9, 22.7. *9-Nonanolactone*: The reaction was complete after 24 h. After distillation, the purity of the lactone was 93%. ^{1}H NMR ($CDCl_3$): δ 4.25 (t, 2H, CH_2O), 2.30 (t, 2H, CH_2C═O), 1.70 (4H, m, CH_2), 1.50 (8H, m, CH_2) ^{13}C NMR ($CDCl_3$): δ. 173.9, 66.1, 34.9, 27.2, 25.8, 24.5, 24.2, 22.9, 20.6. *10-Decanolactone*: The reaction was complete after 6 days; bp = 62−65 °C at 1.3 mbar. ^{1}H NMR ($CDCl_3$): δ 4.15 (t, 2H, CH_2O), 2.32 (t, 2H, CH_2C═O), 1.70 (4H, m, CH_2), 1.34 (10H, m, CH_2). ^{13}C NMR ($CDCl_3$): δ 174.1, 64.7, 35.2, 26.2, 25.4, 25.3, 24.7, 24.1, 22.3, 21.3. *12-Dodecanolactone:* The reaction was stopped after 10 days; bp = 85 °C at 0.28 mbar. ^{1}H NMR ($CDCl_3$): δ 4.15 (m, 2H, CH_2O), 2.35 (m, 2H, CH_2C═O), 1.68 (m, 4H, CH_2), 1.28 (m, 14H, CH_2).^{13}C NMR ($CDCl_3$): δ 174.0, 64.5, 34.6, 27.3, 26.5, 2 × 26.3, 25.3, 25.2, 24.8, 24.4, 24.1.

Novozym 435-Catalyzed Polymerization of Lactones Initiated with Benzyl Alcohol. To prevent water initiation, the eROP of the lactones was carried out in "dry" conditions. To do so, Novozym 435 (∼100 mg) was weighed in an oven-dried flask and placed overnight in a vacuum oven at 50 °C and at ∼25 mbar. A separate beaker with P_2O_5 was placed in the oven as well to ensure drying. The vacuum was released by backfilling the oven with nitrogen. The reaction mixture was prepared by adding the appropriate lactone (10 mmol), 1,3,5-tri-*tert*-butylbenzene (2.5 mmol), benzyl alcohol (0.20 mmol), and freshly distilled toluene (∼5 mL) into an oven-dried Schlenck tube provided with stirring bar. The mixture was stirred at 45 °C under Ar in the presence of 4 Å molecular sieves. The reaction was started when the dried Novozym 435 was added to the mixture, and samples (0.1 mL) were withdrawn from the reaction mixtures at set time intervals. The samples were filtered over a plug of cotton to remove Novozym 435 particles, and the residue was flushed with dichloromethane (∼2 mL) into sample vials, and the concentration of the solution was adjusted to ∼1 mg lactone/mL. The samples were analyzed with GC to determine the conversion of the lactone. When the conversion was >90%, the enzyme was filtered off over a P4 glass filter, and the filtrate was precipitated in MeOH. The resulting white powder was isolated, dried, and analyzed with ^{1}H NMR. *Poly(VL)*: ^{1}H NMR ($CDCl_3$): δ 7.33 (s, *H*−Ph end group), 5.10 (s, CH_2Ph end group), 4.06 (t, CH_2OCO), 3.63 (t, CH_2OH end group), 2.32 (t, CH_2COO), 1.68

Table 1. Overview of Literature Data on the Properties of Lactones[6,14c] [a]

lactone	ring size	bp at 10 Torr[b] [°C]	μ[b] [D]	relative hydrolysis rate[c]	relative polymerization rate[d]	ester conformation[e]
δ-VL	6	97−98	4.22	8460	2780	C
ϵ-CL	7	104−106	4.45	390	370	C
HL	8	80−82	3.70	543	n.a.	C+ little T
OL	9	72−73	2.25	18	23	T + little C
NL	10	86−87	2.01	0.03	n.a.	T
DL	11	100	1.88	0.08	n.a.	T
UDL	12	116	1.86	0.50	1	T
DDL	13	130	1.86	0.90	1.1	T
PDL	16	169	1.86	1.00	1	T
butyl caproate		83	1.79	1.30		T

[a] C = cisoid conformation and T = transoid conformation of ester group; n.a. = not available. [b] Boiling point (bp) and dipole moment (μ) data obtained from ref 14c. [c] Reaction at 0 °C in dioxane/NaOH−water, see ref 14c. [d] Reaction at 100 °C with $Zn(Oct)_2$ as the catalysts, data obtained from ref 6. [e] Data obtained from ref 14c.

(m, CH_2). *Poly(CL):* ^{1}H NMR ($CDCl_3$): δ 7.34 (s, *H*−Ph end group), 5.10 (s, CH_2Ph end group), 4.08 (t, CH_2OCO), 3.63 (t, CH_2OH end group), 2.32 (t, CH_2COO), 1.68 (m, CH_2) 1.41 (m, CH_2). *Poly(HL):* ^{1}H NMR ($CDCl_3$): δ 7.34 (s, *H*−Ph end group), 5.10 (s, CH_2Ph end group), 4.08 (t, CH_2OCO), 3.63 (t, CH_2OH end group), 2.32 (t, CH_2COO), 1.68 (m, CH_2) 1.41 (m, CH_2). *Poly(OL):* ^{1}H NMR ($CDCl_3$): δ 7.34 (s, *H*−Ph end group), 5.10 (s, CH_2Ph end group), 4.08 (t, CH_2OCO), 3.63 (t, CH_2OH end group), 2.32 (t, CH_2COO), 1.68 (m, CH_2) 1.41 (m, CH_2). *Poly(NL)*: ^{1}H NMR ($CDCl_3$): δ 7.36 (s, H−Ph end group), 5.10 (s, CH_2Ph end group), 4.04 (t, CH_2OCO), 3.63 (t, CH_2OH end group), 2.28 (t, CH_2COO), 1.60 (m, 2 × CH_2), 1.30 (m, 4 × CH_2). *Poly(DL):* ^{1}H NMR ($CDCl_3$): δ 7.34 (s, *H*−Ph end group), 5.10 (s, CH_2Ph end group), 4.04 (t, CH_2OCO), 3.63 (t, CH_2OH end group), 2.32 (t, CH_2COO), 1.60 (m, CH_2) 1.28 (m, CH_2). *Poly(UDL)*: ^{1}H NMR ($CDCl_3$): δ 7.34 (s, *H*−Ph end group), 5.10 (s, CH_2Ph end group), 4.04 (t, CH_2OCO), 3.63 (t, CH_2OH end group), 2.32 (t, CH_2COO), 1.60 (m, CH_2), 1.28 (m, CH_2). *Poly(DDL):* ^{1}H NMR ($CDCl_3$): δ 7.34 (s, *H*−Ph end group). 5.10 (s, CH_2Ph end group), 4.04 (t, CH_2OCO), 3.63 (t, CH_2OH end group), 2.32 (t, CH_2COO), 1.60 (m, CH_2), 1.25 (m, CH_2). *Poly(PDL):* ^{1}H NMR ($CDCl_3$): δ 7.34 (s, *H*−Ph end group), 5.10 (s, CH_2Ph end group), 4.08 (t, CH_2OCO), 3.63 (t, CH_2OH end group), 2.32 (t, CH_2COO), 1.60 (m, CH_2), 1.25 (m, CH_2).

General Procedure for the Michaelis−Menten Kinetics in the eROP. In all cases, the amount of Novozym 435 was kept constant at 50 mg while the concentrations of lactone were varied between 2.0, 1.0, 0.5, 0.25, and 0.125 M in toluene. 1,3,5-Tri-*tert*-butylbenzene was added as an internal standard to quantify the conversion with GC. A typical polymerization was conducted as follows: a solution of CL (1.92 g, 8 mmol), toluene (4 mL), and 1,3,5-tri-*tert*-butylbenzene (0.1 g) was heated to 45 °C in an oil bath for 30 min, after which Novozym 435 (50 mg) was added. Samples were taken at appropriate time intervals, filtered over cotton, and analyzed by GC to determine the lactone conversion, from which the initial rate constant could be derived.

Novozym 435-Catalyzed Polymerization of Lactones Initiated with Water. Novozym 435 was dried over P_2O_5 at 1 mbar for 24 h before use. A lactone solution (2 M in toluene) was stirred at 40 °C for 8 h in the presence of 4 Å activated molecular sieves. Subsequently, 4 mL of this solution was added to Novozym 435 (2.5 mg enzyme/mmol lactone) under an argon atmosphere. After 7 days bis(triphenylphosphine)nickel(II) bromide was added to inhibit the enzyme,[13] the enzyme was then removed from the reaction mixture by filtration over a glass filter, and the filtrate was precipitated in cold heptane. The resulting white powder was isolated, dried, and analyzed with ^{1}H NMR, SEC, and DSC. The polyesters were obtained in yields typically around 70%. The ^{1}H NMR spectra of these polymers were identical to the spectra of the polyesters initiated with benzyl alcohol, with the exception that the initiator peaks at 7.34 and 5.10 ppm (benzyl ester) and the end group at 3.63 ppm were absent. The SEC and DSC results are summarized in Table 4.

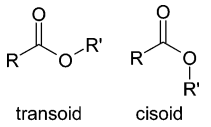

Figure 1. Cisoid and transoid conformation of the ester bond.

Results and Discussion

Synthesis of Lactones. Several lactones such as δ-valerolactone (VL), ϵ-caprolactone (CL), 11-undecanolactone (UDL), 12-dodecanolactone (DDL), and 15-pentadecanolactone (PDL) are commercially available, except 7-heptanolactone (HL), 8-octanolactone (OL), 9-nonanolactone (NL), and 10-decanolactone (DL). The most viable way to synthesize these lactones is via a Baeyer−Villiger oxidation of the corresponding cyclic ketones. We selected *m*-chloroperbenzoic acid (*m*CPBA) as the oxidant of choice despite its relative low reactivity for large cyclic ketones. Cyclooctanone, cyclononanone, cyclodecanone, and cyclododecanone were readily oxidized to their corresponding lactones. During the Baeyer−Villiger oxidation of cycloheptanone, a competing hydrolysis reaction was observed which resulted in moderate yields. In all cases, the lactones were obtained in good to excellent purity.

Properties of Lactones. In the 1950s, Huisgen and coworkers described detailed studies on lactones of different ring sizes to explain their deviating physical properties compared to their linear counterparts.[14] In normal ring lactones (ring size 5−7), high dipole moments provide dipole interactions that rationalize their relatively high boiling points (see Table 1). This high dipole moment is a direct result of the enforced cisoid conformation of the ester bond present in small ring lactones (see Figure 1). In larger ring lactones (starting from the 10-membered ring), the ester bond can adopt a transoid conformation characterized by dipole moments comparable to those of linear esters. The energy difference between the cisoid and transoid conformation amounts to 15 kJ/mol.[14c] The physical differences between lactones adopting cisoid or transoid conformations are also reflected in their propensity to hydrolyze under alkaline conditions. Lactones show fast hydrolysis rates when the ester bond is in a cisoid conformation while the hydrolysis rate of transoid lactones is in the same order of magnitude as their linear counterparts. The transition of the cisoid to transoid conformation is complete with the 9-nonanolactone.

Lipase-Catalyzed Ring-Opening Polymerization of Lactones. Several factors affect the rate of lipase-catalyzed ROP such as temperature, solvent, concentration, choice of initiator, and the water content of the enzyme. To reconcile all these factors, we selected 45 °C as the temperature of choice and performed all polymerizations in toluene at a concentration of

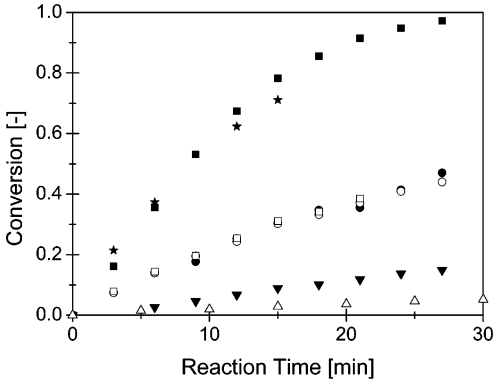

Figure 2. Conversion vs time plots of the Novozym 435-catalyzed ROP of selected lactones: VL (●), CL (○), HL (■), OL (□), DL (△), UDL (▼), DDL (★). Polymerization conditions: 2 mol/L in toluene at 45 °C; M/I ratio = 50/1; initiator = benzyl alcohol.

Table 2. Initial Rate Constants (k_i) and Turnover Numbers (k_{cat}) of EROP of Lactones at 45 °C Catalyzed by Novozym 435

lactone	k_i (R^2)[a] (h^{-1})	[lactone] (mol/L)	$[CALB]_{tot}$ (μmol/L)	k_{cat} [b] (s^{-1})
δ-VL	1.37 (0.99)	1.9	11.5	63.0
ϵ-CL	1.26 (0.99)	1.9	11.7	58.0
HL	5.94 (0.97)	1.6	9.8	270.7
OL	1.43 (0.99)	1.6	13.8	44.2
NL	0.13 (0.99)	1.7	10.0	6.0
DL	0.10 (0.98)	1.4	8.2	4.7
UDL	0.38 (0.99)	1.6	14.3	11.7
DDL	4.91 (0.99)	1.9	11.7	223.0
PDL	2.03 (0.96)	1.9	11.7	89.5
PDL[c]	4.87 (0.97)	2.0	7.5	360.0

[a] R^2 is the error on the linear fit of the ln(1 − conversion) vs time plot. [b] k_{cat} was calculated by $(k_i[\text{lactone}])/(3600[CALB]_{tot})$, where $[CALB]_{tot}$ is the total active CALB concentration. [c] Measured at 60 °C with water as initiator.

2 mol/L. Benzyl alcohol was selected as the initiator to control the molecular weight in a monomer/initiator molar ratio of 50/1. In all cases, Novozym 435 and all reactants and solvents were carefully dried to exclude effects related to the amount of water in the system.[15] For all polymerizations, the overall lactone conversions were plotted as a function of time (see Figure 2), and the first-order rate constants were determined assuming a first-order reaction. The results are summarized in Table 2.

Three regimes can be distinguished in Figure 2: fast reacting monomers such as HL and DDL, slow reacting monomers such as UDL and DL, and monomers with intermediate reaction rates (VL, CL, and OL). Previously, it was suggested that the polarity/hydrophobicity of the lactone was predominantly responsible for its reactivity in lipase-catalyzed ROP.[8] However, the differences we observe here cannot be related to dipole moments only: the difference between UDL and DDL is only 1 methylene group while the ratio in initial rate constants is 13. The same holds, although less pronounced, for HL and CL where the ratio in initial rate constants is 5.

As a result of the polymer's poor solubility, the polymerization of PDL proved to be troublesome at 45 °C and at 2 mol/L: the polymer precipitated during sample taking, and the reaction medium was highly viscous. Therefore, the reaction was repeated at 60 °C. Although the kinetics of this reaction cannot be directly compared to the polymerizations conducted at 45 °C, the initial rate constant of the PDL polymerization is 4.87 h^{-1}, and this shows that PDL polymerizations are also fast.

To make a better comparison between the different polymerizations—the concentrations of Novozym 435 and lactone were not identical in all polymerization reactions (see

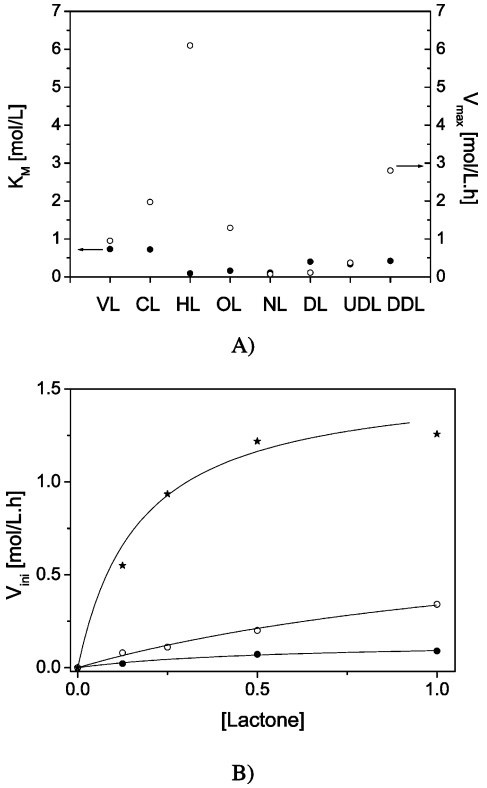

Figure 3. (A) K_M (closed circles) and V_{max} (open circles) as a function of the number of carbons in the lactone ring. The results for PDL are omitted since they were obtained at a higher temperature. (B) Saturation plots of the Novozym 435-catalyzed eROP of DDL (stars), UDL (open circles), and DL (closed circles) measured at 45 °C in toluene.

Table 2)—the turnover number, k_{cat}, was calculated from the initial rate constants. The turnover number varies between ~5 and 270 s^{-1} for the polymerizations conducted at 45 °C, showing that the ratio in rates between the fastest (HL) and slowest (DL) lactone is 57. Interestingly, all lactones studied here are conveniently polymerized with Novozym 435 within reasonable time scales: a conversion of 50% is reached for DDL within 8 min, while it takes around 7 h for DL. When we compare the results of Novozym 435-catalyzed eROP with $Zn(Oct)_2$-catalyzed ROP, the conversion of 50% reached for DDL within 8 min, employing Novozym 435 contrasts sharply with a conversion of ~45% after 160 h at 100 °C with $Zn(Oct)_2$-catalyzed ROP.[6]

Michaelis Menten Evaluation of Lactones. To get more insight into the factors governing the kinetics of lipase-catalyzed ring-opening polymerizations of lactones, the Michaelis–Menten constant K_M and the maximal rate of reaction V_{max} were determined for all lactones studied. The results are summarized in Table 3 and visualized in Figure 3A. From these results, the turnover number, k_{cat}, and the specificity constant, k_{cat}/K_M, were derived (Table 3). The Hanes–Woolf plot was selected to calculate V_{max} and K_M although other plots (Lineweaver–Burke and Eadie–Hofstee) gave similar results (data not shown). Only in the case of HL was the error in the linear fit of the Hanes–Woolf plot ($R^2 = 0.72$) high, and therefore, the results of K_M and V_{max} should be taken only as indicative values. This large error is primarily related to the extremely high reactions rates for HL polymerizations especially at low substrate concentrations (conversion > 50% within 5 min), making the kinetic measurements less reliable. To circumvent solubility problems with the PDL polymerization, we decided to measure the K_M and V_{max} at 60 °C.

Table 3. Michaelis−Menten Constants of Novozym 435 ROP of Lactones at 45 °C

lactone	K_M (mol/L)	V_{max} (mol/(L h))	k_{cat}[a] (s^{-1})	k_{cat}/K_M (L/(mol s))	R^2 [b]
δ-VL	0.73	0.95	35.2	48.2	0.95
ϵ-CL	0.72	1.97	72.9	101.3	0.91
HL	0.09	6.10	225.9	510.3	0.72
OL	0.16	1.29	47.7	298.6	0.99
NL	0.11	0.07	2.6	23.6	0.97
DL	0.40	0.11	4.0	10.2	0.88
UDL	0.33	0.37	13.7	41.5	0.99
DDL	0.42	2.80	103.7	246.9	0.99
PDL[c]	0.31	5.51	204.0	658.9	0.98

[a] $[CALB]_{tot} = 7.5 \times 10^{-6}$ mol/L. [b] R^2 is the error on the linear fit of the Hanes−Woolf plot. [c] Measured at 60 °C.

Table 3 shows that the values for K_M are in the same range (between 0.09 and 0.73 mol/L) for all lactones studied. On the other hand, the values for V_{max} vary between 0.07 and 6.10 mol/(L h) and do not appear to show a trend. The similar values found for K_M are in line with the results of the eROP of selected lactones previously performed with Lipase PF (K_M(CL) = 0.61 mol/L, K_M(UDL) = 0.58 mol/L, K_M(DDL) = 1.1 mol/L, K_M(PDL) = 0.80 mol/L).[6,8] Since Lipase PF is a less active enzyme for lactone polymerizations, the values found for V_{max} of the lactones (V_{max}(CL) = 0.66×10^{-2} mol/(L h), V_{max}(UDL) = 0.78×10^{-2} mol/(L h), V_{max}(DDL) = 2.3×10^{-2} mol/(L h), V_{max}(PDL) = 6.5×10^{-2} mol/(L h)) were significantly lower compared to the values of V_{max} we observe for Novozym 435-catalyzed polymerizations (Table 3).

Values for k_{cat} derived from V_{max} (Table 3) are in good agreement with the values of k_{cat} calculated for the eROP of lactones conducted at ∼2 mol/L (Table 2). This is expected since the benzyl alcohol-initiated polymerizations are conducted well above the K_M of the lactones. Despite the low R^2 values for HL (R^2 = 0.72) in the Hanes−Woolf plot (Table 3), the values calculated for k_{cat} of 225.9 and 270.7 s^{-1} from V_{max} and for the eROP at ∼2 mol/L, respectively, are in good agreement. In the case of DDL the k_{cat} derived from V_{max} (103.7 s^{-1}) is a factor 2 lower than the k_{cat} calculated for the eROP (223.0 s^{-1}). Also in this case, DDL displays fast polymerization kinetics which may lower the accuracy of the data obtained.

To visualize the dramatic differences that arise upon increasing the lactone ring size from an 11- to a 13-membered ring, the saturation plots of DL, UDL, and DDL are shown in Figure 3B: V_{max} decreases from 2.80 to 0.37 and 0.11 mol/(L h) for DDL, UDL, and DL, respectively, while the dipole moment of these lactones is almost the same (Table 1, $\mu \sim 1.86$ D).

Origin of the Differences in Reactivity of Lactones in eROP. Since there is no obvious correlation between a lactone's reactivity and its physical properties (such as dipole moment) in eROP, we decided to take the reactivity of lactones in alkaline hydrolysis reactions as reported by Huisgen as a reference.[14] We are well aware of the mechanistic differences between alkaline hydrolysis reactions and lipase-catalyzed reactions, but data for acid-catalyzed hydrolyses of lactones are, to our knowledge, not available, and the data for $Zn(Oct)_2$-catalyzed ROP of lactones are not complete.[6] The series of lactones we have investigated can be divided into three classes: normal rings (5−7-membered lactone rings), medium-sized rings (8−12-membered lactone rings), and large rings (13- and higher-membered lactone rings).[14c] The reactivity of Novozym 435-catalyzed eROP will be discussed for each of these classes and will be related to the origin of the reactivity differences previously discussed for alkaline hydrolysis reactions and, if applicable, the $Zn(Oct)_2$-catalyzed ROP. To visualize similarities and discrepancies, the V_{max} of eROP and the rates of alkaline hydrolysis of the lactones VL to DDL are compared by normalizing all data with respect to DDL.[16] DDL was selected since it is the first lactone that behaves as a linear ester, and alkaline hydrolyses rates of DDL and PDL were found to be almost identical (Table 1). The results are summarized in Figure 4.

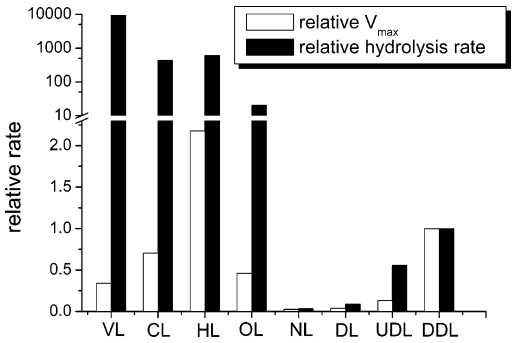

Figure 4. Comparison of the relative rates of alkaline hydrolysis (Table 1, data obtained from ref 14c) and eROP (Table 3) of lactones VL to DDL. All values are normalized with respect to DDL.

5−7-Membered Lactone Rings. Although not discussed in this paper, we observed that γ-butyrolactone is unreactive in Novozym 435-catalyzed eROP, which is in agreement with the thermodynamic stability of the 5-membered lactone ring.[17] VL and CL show moderate polymerization rates in the eROP. This is in sharp contrast to the high polymerization rates of VL and CL by $Zn(Oct)_2$ catalysis and the high rates in alkaline hydrolysis reactions (see Table 1). Huisgen et al. ascribed the high reaction rates for the 6- and 7-membered lactones to the higher energy level of the ground state of a lactone in a cisoid conformation: this results in a lower activation energy and consequently enhances the reaction rate.[14c] In $Zn(Oct)_2$-catalyzed ROP, the high reactivity of VL and CL has been rationalized by the ring strain present in the 6- and 7-membered rings. This strain is partially released in the transition state, resulting in a lower enthalpy of activation compared to those of unstrained lactones.[6]

8−12-Membered Lactone Rings. The reactivity of these medium-sized rings in lactone hydrolysis is predominantly governed by conformational strain (the inability of C−C bonds to attain anti conformations in cyclic compounds) and transannular interactions (repulsion between nonneighboring H atoms) and was studied in detail.[14c,18] In eROP, HL shows a higher reactivity than CL (Figure 4). In analogy to the reactivity of HL in alkaline hydrolysis, this can be explained by the release of transannular interactions during the transition from a trigonal C═O center into a tetragonal center, needed for the activation of the ester in lipase catalysis, thus decreasing the activation energy [14c] OL, on the other hand, has a similar reactivity in eROP as VL and CL, suggesting that transannular interactions in the 9-membered ring are less important. Conformational strain is known to reach a maximum in the 10-membered ring and governs the reactivity of 10−12-membered lactone rings in alkaline hydrolysis.[14c,18] The reason for the low reactivity in NL and DL results from release of the conformational strain by the presence of the trigonal C═O center. Consequently, the resistance of the trigonal C═O center to convert to the tetragonal center needed for activation of the ester in alkaline hydrolysis results in an increase of the activation energy, and a concomitant decrease of reaction rates. It appears from Figure 4 that the trend in the relative rates of eROP in NL, DL, and UDL closely follows the trend of the relative rates of alkaline hydrolysis.

This suggests that conformational strain in these lactones, responsible for the low reactivity in alkaline hydrolyses, indeed plays an important role in the reactivity of NL, DL, and UDL in eROP.

13- and More-Membered Lactone Rings. In $Zn(Oct)_2$-catalyzed polymerizations and alkaline hydrolysis reactions large rings display low reactivities, which is a direct result of the transoid conformation and the resulting lower energy level of the ground state.[14c] Ring strain is almost nonexistent, and these lactones start behaving as open chain esters. In eROP, on the other hand, the large rings polymerize relatively fast. These lactones are highly hydrophobic and have a more linear shape. However, the K_M of all lactones investigated is rather similar, suggesting that the binding abilities of CALB are independent of the ring size.

Discussion. Evidently, there is a striking similarity in the relative rates of alkaline hydrolysis and Novozym 435-catalyzed eROP starting from the 10-membered lactone rings (see Figure 4). In these lactones, the ester groups completely adapt a transoid conformation (Table 1). This suggests that lactone reactivity in eROP starting from the 10-membered ring is predominantly governed by conformational effects similarly to the lactone reactivity in alkaline hydrolyses reactions. On the other hand, there is a huge discrepancy between the reactivity of lactones having a cisoid conformation in alkaline hydrolysis reactions and in eROP. Although the general trend is similar—rate(CL) < rate(HL) > rate(OL)—the relative reactivity differs orders of magnitude, as evidenced by Figure 4. In fact, the comparison between alkaline hydrolysis reactions and eROP illustrates this: while the ratio in reactivity between VL and DDL amounts to ~9400 in alkaline hydrolysis reactions, the ratio in reactivity between VL and DDL is only ~3 in eROP. As a result, the difference in activation energy between VL and DDL for an alkaline hydrolysis is around 18 kJ/mol, while the difference in activation energy for eROP is around −3 kJ/mol. The question arises why lactones in a cisoid conformation are less reactive in eROP than is expected on the basis of their intrinsic reactivity. At the moment, we do not have an adequate explanation for this observation, but several factors may play a role. First, in an alkaline hydrolysis reaction, the C═O is freely accessible for the hydroxide anion irrespective of the conformation of the ester bond. In eROP, the attack of the serine alcohol on the C═O is by definition taking place in a sterically confined space.[19] It is very well possible that the conformation of the ester group (cisoid or transoid) places severe restrictions on the accessibility of the C═O in the enzyme's active site. Moreover, the stabilization of the transition state by additional H-bonds in the oxyanion hole may be influenced by the ester conformation of the lactone. Second, besides having high dipole moments, it has also been shown that 6- and 7-membered ring lactones have a higher basicity than large ring lactones.[20] Moreover, the H-bond strength of these lactones is significantly higher than that of large ring lactones.[21] In an environment where H-bonds are crucially important to stabilize the transition state, and polarity and pH may have a large effect, this may have significant consequences for the reactivity of lactones.

Properties of Polyesters Obtained by Novozym 435-Catalyzed eROP. Novozym 435 is a versatile and unique catalyst that can readily polymerize lactones irrespective of their ring size. Since there is no other catalyst available that can efficiently polymerize such a wide range of lactones at mild temperatures, we synthesized polyesters of reasonable molecular weight to conduct a preliminary characterization with differential scanning calorimetry (DSC) and size exclusion chromatography (SEC). By using only small amounts of Novozym 435 to limit the amount of water, we expected to obtain polymers with a molecular weight of around 20 000 g/mol. All polymers were precipitated in heptane before characterization and were obtained as white powders.

Table 4. Properties of Polyesters Obtained via Novozym 435-Catalyzed eROP at 45 °C[a]

polyester	M_n (g/mol)	PD	T_m (°C)[d]	ΔH (J/g)[d]
poly(VL)	16 300[b]	4.1[b]	57.5	62.2
	19 000[c]	4.0[c]		
poly(CL)	5200[b]	3.3[b]	54.4	72.7
	10 000[c]	2.2[c]		
poly(HL)	23 600[b]	2.8[b]	65.2	88.7
poly(OL)	20 700[b]	2.7[b]	69.8	80.1
poly(NL)	16 000[b]	2.1[b]	70.1	78.5
poly(DL)	20 000[b]	3.2[b]	77.0	99.6
poly(UDL)	12 000[b]	2.8[b]	83.1	98.0
poly(DDL)	10 600[b]	3.7[b]	84.8	125.4
poly(PDL)	6 600[b]	5.5[b]	92.8	119.7

[a] All molecular weights are relative to PS standards. [b] Measured in ODCB at 80 °C. [c] Measured in THF. [d] Determined from the second heating run.

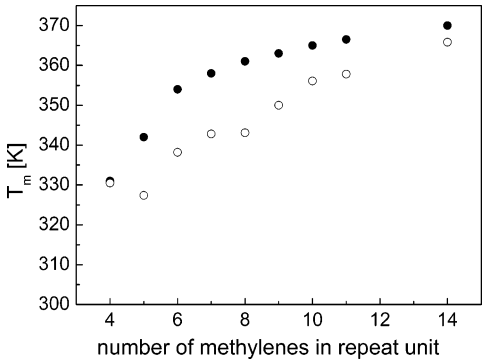

Figure 5. Melting temperature T_m as a function of the number of methylenes in the repeat unit of the polyesters synthesized by eROP. Values determined from the second heating run (open circles) and reported by Lebedev et al. (closed circles).

SEC traces were measured in ODCB at 80 °C to anticipate on the poor solubility of poly(PDL) in $CHCl_3$ at room temperature.[10] Poly(VL) and poly(CL) were also measured in THF. The results are summarized in Table 4. Except for poly(CL) and poly(PDL), the M_n values measured in ODCB are around 10 000−20 000 g/mol. The presence of a low molecular weight shoulder in most SEC traces accounts for the rather high polydispersities, varying from 2 to 5. We presume that this low molecular weight shoulder arises either from the breakdown of the polymer during filtration or from cyclic species, which are always present in the MALDI-TOF spectra.

Preliminary DSC studies of the polyesters show an increase of the melting temperature and melting enthalpy with increasing number of methylene units (see Table 4). The results are visualized in Figure 5. Although consistently lower, the experimental melting temperatures nicely correlate with the melting temperatures that were predicted by Lebedev and coworkers for this series of polyesters.[22] The consistent discrepancy between the experimental and theoretical values can be rationalized by considering that the melting temperatures reported here are not equilibrium melting temperatures. Moreover, the low molecular weight fraction visible in the SEC traces may be responsible for a lowering of the melting temperature. An indication for the importance of the latter can be found when comparing data of poly(PDL) reported by Gross et al. (T_m = 97 °C, M_n = 86 400 g/mol, PD = 2.3) with our data on poly(PDL) (T_m = 92.8 °C, M_n = 6600 g/mol, PD = 5.5).[23] A higher M_n and a lower polydispersity clearly leads to an increase

of the melting temperature. As predicted, no odd−even effect is observed in this series of polyesters, which is in contrast to the [*n*]-polyamides and the [*n*]-polyurethanes.[24] A more detailed study on the thermal behavior of this series of polyesters is in progress.

Conclusions

Novozym 435-catalyzed ring-opening polymerizations of lactones of varying ring sizes (6- to 13- and the 16-membered ring) demonstrate fascinating differences in their polymerization rates. These differences cannot be related to variations in physical properties, such as the dipole moment, of the lactones only. For example, 10-decanolactone, 11-undecanolactone, and 12-dodecanolactone show dipole moments of around 1.9 D, but the initial rate constants of their Novozym 435-catalyzed polymerization were found to be 0.10, 0.38, and 4.91 h^{-1}, respectively. The Michaelis−Menten constants K_M and V_{max} were measured for all lactones, and this showed that the K_M was relatively independent of the ring size, suggesting similar affinities of the lipase for all lactones, while no obvious trend could be discerned for V_{max}. However, conformational strain and transannular interactions present in medium ring lactones (ring size 8−12) affect the reactivity of these lactones in lipase catalysis in a similar way as was previously described for the alkaline hydrolysis reactions of lactones, rationalizing the low reactivity of the 10−12-membered rings and the high reactivity of the 8-membered ring. To our surprise, there is a large discrepancy in lipase-catalyzed ring-opening polymerizations compared to the alkaline hydrolysis reactions with regard to lactones possessing a cisoid conformation of the ester bond. While the ratio in rates between VL and DDL in an alkaline hydrolysis is 9400, the ratio in rates using Novozym 435 is only 3. In fact, in the latter case lactones possessing a cisoid conformation are less reactive than large ring lactones in a transoid conformation. Several factors may play a role here such as effects of basicity and dipole moment of the lactone and steric interactions with surrounding amino acid residues in the active site, but at the moment it remains concealed how the relative contributions of each of these factors are. Nevertheless, Novozym 435 is a unique catalyst that enables the synthesis of polyesters with an increasing number of methylenes in their repeat unit. The polyesters can be prepared in reasonable molecular weights (10 000−20 000 g/mol) and show an increase in melting temperature and melting enthalpy with an increasing number of methylenes in the repeat unit. Future research will be aimed at elucidating the factors that determine the reactivity difference in lactones possessing cisoid and transoid conformations in eROP.

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(16) Note that the absolute reaction rates (v) of DDL in alkaline hydrolysis and eROP are completely different: v(eROP) = 9.3 mol/(L h) at 45 °C (this work) and v(hydrolysis) = 0.048 mol/(L h) at 50 °C using 1 equiv of base (ref 14c).
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