Lipase-Catalyzed Synthesis of an Epoxy-Functionalized Polyester from the Suberin Monomer *cis*-9,10-Epoxy-18-hydroxyoctadecanoic Acid

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Introduction

The synthesis of polymers with tailored reactive functionalities plays an important role in the development of various materials for industrial and biomedical applications. In addition, there is a need to develop chemistry that is based on the use of biodegradable and renewable resources.[1]

Appreciable amounts of natural polyesters occur in higher plants as structural components, for example, cutin in the cuticle that covers the aerial parts of plants and suberin in the cork cells present in the periderm of woody plants. Common aliphatic monomers in these polyesters are long-chain ω-hydroxyalkanoic acids and α,ω-alkanedioic acids, usually together with small amounts of substituted ω-hydroxyalkanoic acids such as *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**).[2] The monomer composition is usually complex and differs between plant species, and this complexity probably explains why the use of cutin and suberin as a commercial source of "green" chemicals has not been well explored. One exception is the suberin in the outer bark of birch, where *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) is the principal monomer amounting to about 100 g/kg dry outer bark and 40% of the aliphatic suberin monomers in *Betula verrucosa*.[3] This epoxy acid can be isolated in high yield from alkali-hydrolyzed outer bark, a low value byproduct in the forest industry mainly used as fuel, by extraction followed by selective precipitation by acidification.[4,5]

Lipase-catalyzed polymerization may sometimes allow straightforward synthesis strategies for polyesters, which are difficult to prepare by more conventional polymerization processes, and has been used, for example, for the preparation of polyesters from epoxy-containing monomers. Activation of the substrate is often used to increase the yield and molecular weight in the polymerization. Common examples are activation of diacids by esterification and hydroxy acids by lactonization.[6−8] However, this requires further reaction steps and must be considered as a less attractive alternative for commercial explorations.

Recently, it was reported that suberin-related long-chain linear 9,10-epoxidized C_{18}, C_{20}, and C_{26} α,ω-dicarboxylic acid dimethyl esters can be polymerized with diols using *Candida antarctica* lipase B (Novozym 435), still keeping the epoxy groups intact.[7] It has also been shown that linear C_{10} to C_{16} aliphatic ω-hydroxy acids can be polymerized efficiently without activation.[9]

In this study, we report the Novozym 435-catalyzed condensation polymerization of *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) isolated from the outer bark of *Betula verrucosa* to form poly(9,10-epoxy-18-hydroxyoctadecanoic acid) (**2**) and compare the reaction with the corresponding polymerization of 10-hydroxydecanoic acid (Figure 1).

Experimental Section

Materials. 10-Hydroxydecanoic acid was purchased from Sigma-Aldrich and used as received. Immobilized *Candida antarctica* lipase B (Novozym 435, specific activity 7000 PLU/g) was provided by Novozymes (Denmark). Acetonitrile, dioxane, methanol, 2-propanol, tetrahydrofuran, and toluene of reagent grade were purchased from Merck (Darmstadt, Germany) and used without further purification. Solvents for the enzymatic polymerizations were stored over activated molecular sieves (Merck 4 Å).

Isolation of *cis*-9,10-Epoxy-18-hydroxyoctadecanoic Acid (1). The epoxy acid (**1**) was isolated as previously described from outer birch bark (*Betula verrucosa*) and recrystallized from 2-propanol to give a light yellow powder.[3−5] The purity of the epoxy acid used in polymerizations was >95% (Glc-MS) and the mp 79−81 °C with the main contaminant probably being the corresponding 2-propyl ester formed by transesterification during the isolation procedure.[4] After repeated purification, the mp was 84−85 °C and $[\alpha]_D$ 0° (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ: 1.2−1.8 (26 H, bm, $-CH_2-$), 2.3 (2H, t, J = 7.4 Hz, $-COCH_2-$), 2.9 (2H, bs, $-CH-$ *cis*-epoxide), 3.6 (2H, t, J = 6.5 Hz, $-CH_2OH$).

^{13}C NMR ($CDCl_3$) δ: 178.48 (C=O, C-1), 62.87 ($-CH_2OH$, C-18), 57.45 ($-CH-$, *cis*-epoxide, C-9 and C-10), 24.82−34.11 (14 C, $-CH_2-$).

Polymerization in Solvent. A mixture of *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) (40 mg, 130 mM) and Novozym 435 (10 mg) was added to capped reaction vials (5 mL). Toluene, dioxane, or acetonitrile (400 μL) was added, and the sample was placed in a constant temperature oil bath. Reference reactions without addition of lipase were also performed. The vials were shaken at 140 rpm for different periods of time at 75 °C. Activated molecular sieves (20 mg Merck 4 Å) were added to some of the vials to remove water. To compare the relative reactivity of epoxy acid (**1**) with other long-chain ω-hydroxy acids, a series of experiments using 10-hydroxydecanoic acid was carried out in the same way with toluene as solvent.[9,10]

Polymerization was performed at a larger scale using toluene as solvent followed by precipitation from methanol to obtain samples for product characterization. Novozym 435 (0.5 g) and molecular sieves (0.5 g) were mixed with *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) (2.0 g) in a capped reaction vial. Toluene (20 mL) was added, and the sample was placed in a constant temperature oil bath with magnetic stirring at 75 °C. After 2 days, the reaction mixture was filtrated, and the solvent was evaporated. The residue was dissolved in tetrahydrofuran (5 mL) and precipitated in cold methanol (50 mL) to give the polyester (**2**) (1.1 g) as a white powder.

^{1}H NMR ($CDCl_3$) δ: 1.2−1.8 (26 H, bm, $-CH_2-$), 2.3 (2H, t, J = 7.5 Hz, $-COCH_2-$), 2.9 (2H, bs, $-CH-$ *cis*-epoxide), 3.6 (t, J = 7.2 Hz, $-CH_2OH$ end group), 4.1 (2H, t, J = 6.5 Hz, $-CH_2O-$).

^{13}C NMR ($CDCl_3$) δ: 173.92 (C=O, C-1), 64.46 ($-CH_2O-$, C-18), 57.24 ($-CH-$, *cis*-epoxide, C-9 and C-10), 26.04−34.47 (14 C, $-CH_2-$).

The consumption of monomers and the formation of polyesters were analyzed by GPC and MALDI-TOF MS (see below).

Bulk Polymerizations. *cis*-9,10-Epoxy-18-hydroxyoctadecanoic acid (**1**) (200 mg, 640 mM) was melted in a constant temperature oil bath with a magnetic stirrer at 85 °C in capped (in the presence of 50 mg molecular sieves) or uncapped vials (5 mL) when performing reactions without molecular sieves. After the monomer (**1**) was melted, Novozym 435 (50 mg) was added. Samples were drawn at different periods of time for analysis by GPC and MALDI-TOF MS.

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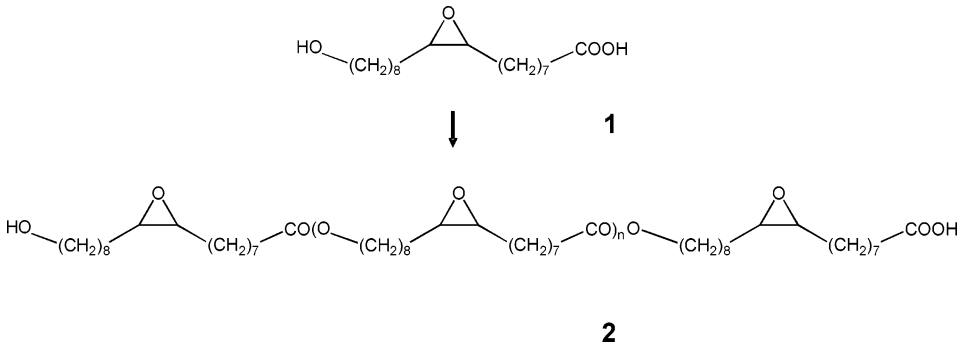

Figure 1. *Candida antarctica* lipase B-catalyzed polymerization of *cis*-9,10-epoxy-18-hydroxy-octadecanoic acid (**1**) to give the corresponding epoxy-functionalized polyester (**2**).

To compare the relative reactivity of the epoxy acid, a series of bulk polymerizations using 10-hydroxydecanoic acid was carried out in the same way.[9]

Instrumental Methods. *Matrix-assisted laser desorption/ionization-time-of-flight mass spectroscopy* (MALDI-TOF MS) was used to estimate the initial progress of the polymerization.

Mass spectra were obtained on a Hewlett-Packard G2025 A LD-TOF system. Acetonitrile was used as solvent for the matrix 2.5-dihydroxybenzoic acid (50 mg/mL). Mixtures of matrix and samples were prepared in the volume ratio 1:1. An aliquot (0.5 μL) was applied to the sample probe, the solvent was evaporated by vacuum, and the probe was inserted into the mass spectrometer. Spectra were taken in the positive ion mode.

Gel Permeation Chromatography (GPC). The average molecular weights of the polymer products were determined by GPC. The GPC apparatus was calibrated using polystyrene standards (Polymer Laboratories) in a molecular range from 580 to 19 880 Da. An aliquot (150 μL) from the reaction vial was added to tetrahydrofuran (2 mL) and filtrated through a 0.45 μm PVDF membrane, before injection into the GPC system. The GPC system was equipped with a Scantec 625 HPLC pump connected to a Waters 410 differential refractometer and a Rheodyne 7125 injector (20 μL loop). Three columns from Waters (Styragel HR2 7.8 $\times$ 300 mm, Styragel HR1, and Ultrastyragel 10^4) connected in series were used for separation. Tetrahydrofuran was used as eluent at a flow rate of 0.8 mL/min.

Nuclear Magnetic Resonance (*NMR).* ^{1}H and ^{13}C NMR spectra of the isolated *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) and the polyesters (**2**) formed in the reactions were obtained with a Bruker DPX 300 system at room temperature at a magnetic field strength of 7.04 T (300 MHz ^{1}H resonance frequency) using $CDCl_3$ as solvent and tetramethylsilane as reference.

Results and Discussion

cis-9,10-Epoxy-18-hydroxyoctadecanoic acid (**1**), the major suberin component in the outer birch bark of *Betula verrucosa*, was isolated in a nearly quantitative yield as a slightly yellow powder as previously described.[3−5] The epoxy acid (**1**) is probably a mixture of the 9*R*,10*S* and 9*S*,10*R* enantiomers as suggested from studies on formation of **1** in relation to the cutin biosynthesis, and the low optical rotation $[\alpha]_D$ 0° indicates a racemic or nearly racemic mixture.[11]

***Candida antarctica* Lipase B-Catalyzed Synthesis of Epoxide-Containing Polyesters (2).** *Candida antarctica* lipase B immobilized on a macroporous acrylic acid resin (Novozym 435) was chosen as catalyst as it has been shown to be a highly efficient catalyst for polymerization of long-chain aliphatic ω-hydroxy acids.[9] Recently, it was also shown that suberin-related 9,10-epoxidized long-chain α,ω-dicarboxylic acid dimethyl esters can be polymerized with diols without affecting the epoxy functions using Novozym 435 as catalyst and diphenyl ether as solvent.[7] Epoxidized fatty acids have also been polymerized with Novozym 435 as catalyst in one-pot reactions together with divinyl sebacate and glycerol to prepare epoxy-functionalized polyesters in good yields.[8] A fairly high charge of Novozym 435 (25 wt %) was used to minimize side-reactions involving the epoxide ring (see below).

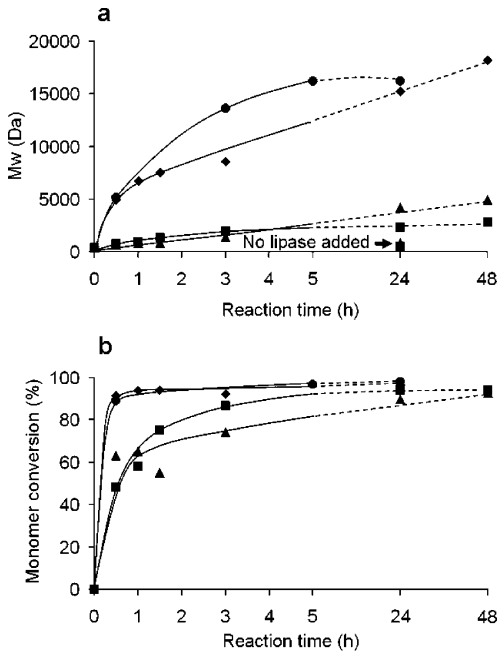

Figure 2. Novozym 435-catalyzed polycondensations of *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) in solvent (75 °C, epoxy acid 40 mg, enzyme 10 mg, solvent 400 μL, molecular sieves 20 mg) and bulk (85 °C, epoxy acid 200 mg, enzyme 50 mg, molecular sieves 50 mg). (a) Extent of chain growth as a function of time and (b) monomer conversion. Investigated solvents: toluene (◆), acetonitrile (■), dioxane (▲), and monomer melt (●).

Effect of Solvent. Some common solvents used in lipase-catalyzed polymerizations are compared to bulk polymerization in Figure 2. The temperature used for the solvent polymerizations was 75 °C. For the bulk polymerizations, a higher temperature of 85 °C was selected to melt the epoxy acid (mp 79−81 °C). The four solvents investigated, acetonitrile, dioxane, toluene, and monomer melt (bulk polymerization), showed similar trends with regard to molecular weight and monomer conversion as has been observed earlier in Novozym 435-catalyzed ring-opening polymerization of ϵ-caprolactone and ω-pentadecalactone.[12,13] The highest molecular weight (M_w 20 000 Da, 68 h reaction time) was obtained when toluene was used as solvent. However, a nearly as high molecular weight (M_w 16 000 Da) was obtained in the bulk polymerizations at a much shorter reaction time (5 h). This was the maximum molecular weight analyzed for the bulk polymerization because

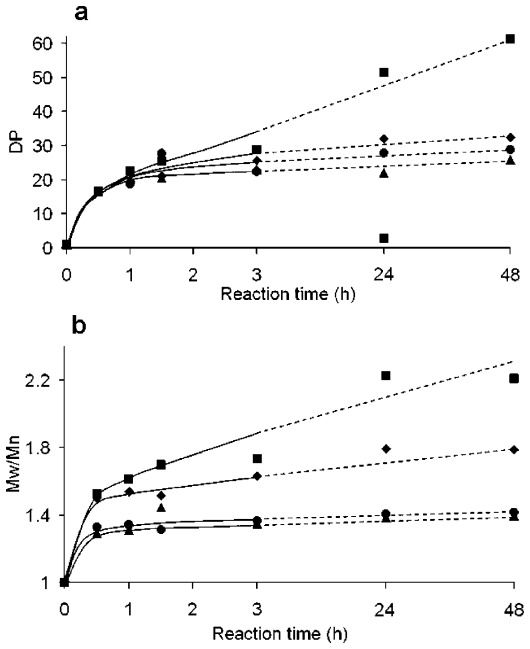

Figure 3. Degree of polymerization (DP) and polydispersity index (M_w/M_n) in Novozym 435-catalyzed polycondensations of *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) and 10-hydroxy-decanoic acid, respectively, in toluene (75 °C, hydroxy acid 40 mg, enzyme 10 mg, solvent 400 μL) with and without molecular sieves (20 mg) present. *cis*-9,10-Epoxy-18-hydroxyoctadecanoic acid with (■) and without (◆) molecular sieves present. 10-Hydroxydecanoic acid with (●) and without (▲) molecular sieves present.

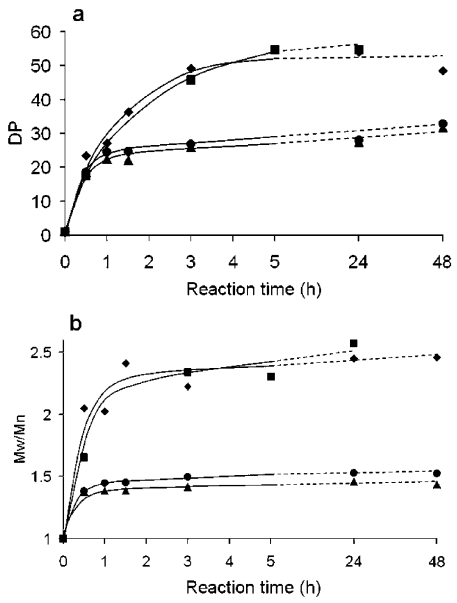

Figure 4. Degree of polymerization (DP) and polydispersity index (M_w/M_n) in Novozym 435-catalyzed polycondensations of *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) and 10-hydroxy-decanoic acid, respectively, in bulk (85 °C, hydroxy acid 200 mg, enzyme 50 mg) with or without molecular sieves (50 mg) present. *cis*-9,10-Epoxy-18-hydroxyoctadecanoic acid with (■) and without (◆) molecular sieves present. 10-Hydroxydecanoic acid with (●) and without (▲) molecular sieves present.

at reaction times longer than 24 h, the polymer formed an insoluble gel probably due to side reactions involving cross-linking by the epoxide group.

The monomer consumption was similar in the investigated polymerizations. Initially, there was a fast consumption during the first hours, which finally leveled off at about 95% conversion. The limit at 95% conversion agrees well with the purity of the starting material. Because the monomer shows a fast initial consumption, but the molecular weights continue to increase at longer reaction times, especially when toluene was used as solvent and in the bulk reaction, it suggests condensation and transesterification of oligomer and polymer chains to be important routes for the generation of high molecular weight products.[10]

Effect of Monomer Concentration and Water Removal Procedure. Polymerizations with toluene as solvent and in bulk were also conducted with 10-hydroxydecanoic acid as substrate to compare the influence of monomer concentration and water removal procedure and the relative reactivity of the *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) with previously investigated long-chain ω-hydroxy acids.[10] 10-Hydroxydecanoic acid was chosen for the comparison because it recently was shown to have the same reactivity as the corresponding 12- and 16-carbon straight chain hydroxy acids under similar reaction conditions, but was simpler to analyze by GPC and MALDI-TOF MS in the systems used by us.[9,14] All of the condensations with toluene as solvent and the bulk polymerizations in the presence of molecular sieves as drying agent were performed in capped vials. The bulk polymerizations without molecular sieves were conducted in uncapped vials. The increase in degree of polymerization (DP) against reaction time and the polydispersity index (M_w/M_n) is shown in Figure 3. The highest DP (69) and M_w/M_n (2.2) were obtained with toluene as solvent for the *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) in the presence of molecular sieves. The final build-up in DP was a relatively slow process, indicating a slow condensation of oligomer and polymer units in combination with transesterification, which is also supported by the higher M_w/M_n in this reaction.[10] Recently, enantioselective polymerization of bis-(2,2,2-trichloroethyl) *trans*-3,4-epoxyadipate with 1,4-butanediol has been performed using the porcine pancreatic lipase as catalyst.[6] It should be noted that the polymerizations of the epoxy acid (**1**) isolated from birch outer bark described here did not show any sign of enantioselectivity even though **1** probably is a mixture of the 9*R*,10*S* and 9*S*,10*R* enantiomers.[11]

The bulk polymerization of *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) proceeded rapidly, giving a slightly lower final DP (54) and somewhat higher polydispersity index (M_w/M_n 2.5) as compared to when toluene was used as solvent in the presence of molecular sieves, Figure 4. However, for the bulk polymerization, there was no difference in molecular weight and polydispersity index irrespective of molecular sieves being present or not. The slightly higher polydispersity index (M_w/M_n 2.5) in the bulk polymerization as compared to polymerization with toluene as solvent (M_w/M_n 2.2) is similar to what has been found in corresponding ω-decalactone polymerizations. As compared to 10-hydroxy-decanoic acid, the polymerization of *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) resulted in all cases in higher DP and M_w/M_n.

Characterization of Products. Positive ion MALDI-TOF MS spectra were recorded for products larger than 600D because the range below was dominated by peaks from matrix. Figure 5 shows a mass spectrum of the products from a 30-min polymerization of *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) in toluene. In the mass spectrum, there are two product distributions, both with a repeat unit of 296 D. The first minor peak distribution with a very low intensity indicates the formation of small amounts of Na^+-cationized cyclic oligomers in the initial phase of the reaction and extends over the mass

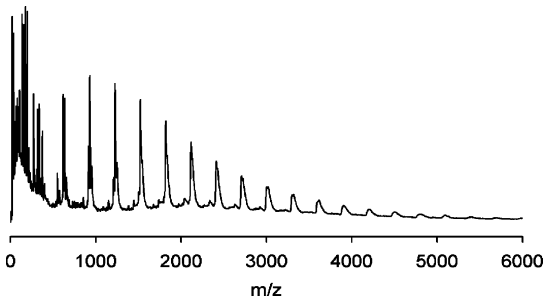

Figure 5. MALDI-TOF MS spectrum of the products from a 30-min polymerization of *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) in toluene catalyzed by *C. antarctica* lipase B.

range 618−3300 D. The second major peak distribution represents Na^+-cationized oligomers and extends over the mass range 636−6000 D.

The ^{1}H NMR spectra of the polyester **2** purified by precipitation from methanol show that in the polyester the *cis*-epoxy group at δ 2.9 ppm is intact.[15] The DP determined from integration of the ^{1}H NMR spectrum (δ 3.6 ppm $-CH_2-OH$ end group) was 30, to be compared with DP 32 as determined by GPC.

Conclusion

Immobilized *Candida antarctica* lipase B (Novozym 435) is an efficient catalyst for the polycondensation of *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) to give epoxy-functionalized polyesters with high molecular weights. The polycondensation performed in toluene in the presence of molecular sieves gave the polyester with the highest molecular weight (M_w 20 000, reaction time 68 h, M_w/M_n 2.2). A nearly as high molecular weight (M_w 15 000, M_w/M_n 2.2) at a much shorter reaction time (3 h) was obtained by bulk polymerization in an open vial without any drying agent present. The performed polycondensations did not show any signs of enantioselectivity even though *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (**1**) isolated from birch outer bark probably is a mixture of the 9*R*,10*S* and 9*S*,-10*R* enantiomers.

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