

A Cutinase with Polyester Synthesis Activity

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Supporting Information

Experimental Procedures and Results

1. Materials

Diacids (succinic acid, adipic acid, suberic acid, and sebacic acid), diols (cyclohexane-1,4-dimethanol, 1,8-octanediol, 1,6-hexanediol and 1,4-butanediol), and lactone (ω -pentadecalactone) were purchased from Aldrich Chemical Co. in the highest available purity and used as received. ϵ -Caprolactone, a gift from Union Carbide, was dried over calcium hydride and distilled under reduced pressure in a nitrogen atmosphere. *Humicola insolens* cutinase was a gift from Novozymes (Bagsvaerd, Denmark). Lewatit OC VOC 1600 was received as a gift from Rohm and Haas.

2. Instrumental Methods

Nuclear Magnetic Resonance (NMR). NMR spectra were recorded in CDCl₃ using a Bruker DPX 300 at 300 (¹H) and 75 (¹³C) MHz. Chemical shifts are reported relative to TMS (δ 0.00) and coupling constants (*J*) are given in Hz. Polymerization of lactones was monitored by ¹H NMR to determine monomer conversion.

Gel Permeation Chromatography (GPC). The number and weight average molecular weights (*M_n* and *M_w*, respectively) were determined by size exclusion chromatography using a Waters 510 pump, a 717 plus autosampler, and a Wyatt Optilab DSP interferometric refractometer coupled to 500, 10³, 10⁴, and 10⁵ Å Ultrastaygel columns in series. Trisec GPC software version 3 was used for calculations. THF (CHCl₃ for PPDL) was used as eluent with a flow rate of 1.0 mL min⁻¹ at 35 °C. Molecular weights were determined on the basis of a conventional calibration curve generated by narrow molecular weight polystyrene standards obtained from Aldrich Chemical Company.

Differential Scanning Calorimetry (DSC). Calorimetric analysis was performed on a TA Instrument DSC 2920 Differential Scanning Calorimeter. After a first heating run at 10 °C min⁻¹ to 120 °C, the sample was cooled at 10 °C min⁻¹ to -85 °C and then heated from -80 °C to 120 °C at 5 °C min⁻¹ unless otherwise specified (Table S1).

3. Procedures

Immobilization of Humicola insolens cutinase

Humicola insolens cutinase was immobilized on Lewatit OC VOC 1600. The resin (700 mg) was first activated with ethanol (10 mL) and dried under vacuum for 60 min to remove traces of ethanol. The resin was further washed with phosphate buffer (pH 7.8, 0.1 M, 3 x 10 mL). Cutinase (used as received, 1.7 mg mL⁻¹, 25 mL) was added to the resin and incubated with gentle shaking (100 rpm) at 4 °C for 72 hrs. Remaining supernatant was removed by centrifugation (10 000 rpm, 5 min, 15 °C). The cutinase loaded resin was freeze dried and the dry weight determined (**W**). The protein contents of supernatants before (**P1**) and after immobilization (**P2**) were determined using the bicinchoninic acid method. The protein loading was calculated as mg protein g⁻¹ of resin = (**P1** – **P2**)/**W**. The enzyme loading was 60 mg protein g⁻¹ or 6 % by wt.

Method A: General Procedure for Polycondensation between Diols and Diacids

Diol (1 mmol) and diacid (1 mmol) were heated to 100 °C to obtain a monophasic solution of reactants and the temperature was then reduced to the reaction temperature (50 °C to 90 °C). Enzyme beads were added to the reaction so that the ratio (w/w) of enzyme to total monomer was 1% and vacuum (10 mm of Hg) was applied 1 hr after addition of the immobilized cutinase. After 48 hrs the reaction was terminated by adding THF (2 mL) and filtration to remove enzyme beads. Solvent was stripped by rotoevaporation and remaining volatiles were eliminated by thorough drying in a vacuum oven. The isolated products were analyzed by NMR (¹H & ¹³C), GPC, and DSC. Conversion was determined by GPC.

Method B: General Procedure for Ring-Opening Polymerization of Lactones

Lactone (1 g), toluene (2 mL, for reactions not run in bulk) and immobilized enzyme (0.1 % w/w enzyme to total weight of monomer) was added under nitrogen atmosphere in a parallel

reactor (Argonaut Advantage Series 2050). The reactions were stirred magnetically while maintaining reactions at pre-determined temperatures (50 °C to 90 °C). After 24 hrs, reactions were terminated by adding CHCl_3 (20 mL), filtration to remove the catalyst beads and stripping the solvent by roto-evaporation. The product obtained was dried in a vacuum oven and analyzed by NMR (^1H & ^{13}C), GPC and DSC. GPC was recorded using THF as the eluent for poly(ϵ -caprolactone) and CHCl_3 as the eluent for poly(ω -pentadecalactone). Monomer conversion to polymer was measured by ^1H NMR.

4. Polymer characterization

Poly(butylene adipate)

Synthesis was by Method A using 1,4-butanediol (90 mg, 1 mmol) and adipic acid (146 mg, 1 mmol) as monomers.

^1H NMR (CDCl_3): δ 1.66-1.93 (8H, m), 2.33-2.54 (4H, m), 4.09 (4H, t, $J = 6.6$ Hz)

^{13}C NMR (CDCl_3): δ 24.8, 25.7, 34.2, 64.2, 173.7

Poly(hexylene adipate)

Synthesis was by Method A using 1,6-hexanediol (118 mg, 1 mmol) and adipic acid (146 mg, 1 mmol) as monomers.

^1H NMR (CDCl_3): δ 1.33-1.44 (4H, m), 1.50-1.77 (8H, m), 2.26-2.39 (4H, m), 4.06 (4H, t, $J = 6.6$ Hz)

^{13}C NMR (CDCl_3): δ 24.8, 26.0, 28.9, 34.3, 64.7, 173.8

Poly(octylene adipate)

Synthesis was by Method A using 1,8-octanediol (146 mg, 1 mmol) and adipic acid (146 mg, 1 mmol) as monomers.

^1H NMR (CDCl_3): δ 1.25-1.40 (8H, m), 1.52-1.72 (8H, m), 2.26-2.37 (4H, m), 4.05 (4H, t, $J = 6.6$ Hz)

^{13}C NMR (CDCl_3): δ 24.8, 26.2, 29.0, 29.5, 34.3, 64.9, 173.8

Poly(cyclohexane dimethylene adipate)

Synthesis was by Method A using cyclohexane-1,4-dimethanol (144 mg, 1 mmol) and adipic acid (146 mg, 1 mmol) as monomers.

^1H NMR (CDCl_3): δ 0.96-1.03 (4H, m), 1.35-1.81 (10H, m), 2.33 (4H, t, $J = 6.3$ Hz), 3.89 (3H, d, $J = 6.5$ Hz) *trans*, 3.99 (1H, d, $J = 7.2$ Hz) *cis*

^{13}C NMR (CDCl_3): δ 24.8, 29.2, 34.3, 37.4, 69.7, 173.8

Poly(cyclohexane dimethylene suberate)

Synthesis was by Method A using cyclohexane-1,4-dimethanol (144 mg, 1 mmol) and suberic acid (174 mg, 1 mmol) as monomers.

^1H NMR (CDCl_3): δ 0.96-1.04 (4H, m), 1.32-1.81 (14H, m), 2.30 (4H, t, $J = 7.4$ Hz), 3.89 (3H, d, $J = 6.5$ Hz) *trans*, 3.98 (1H, d, $J = 7.2$ Hz) *cis*

^{13}C NMR (CDCl_3): δ 25.2, 29.2, 29.3, 34.6, 37.4, 69.6, 174.2

Poly(cyclohexane dimethylene sebacate)

Synthesis was by Method A using cyclohexane-1,4-dimethanol (144 mg, 1 mmol) and sebacic acid (202 mg, 1 mmol) as monomers.

^1H NMR (CDCl_3): δ 0.97-1.04 (4H, m), 1.30-1.82 (18H, m), 2.29 (4H, t, $J = 7.4$ Hz), 3.89 (3H, d, $J = 6.5$ Hz) *trans*, 3.98 (1H, d, $J = 7.2$ Hz) *cis*

^{13}C NMR (CDCl_3): δ 25.4, 29.3, 29.5, 29.6, 34.7, 37.5, 69.6, 174.3

Poly(ϵ -caprolactone)

Synthesis was by Method B and the monomer was ϵ -caprolactone (1 g, 8.76 mmol).

^1H NMR (CDCl_3): δ 1.35-1.43 (2H, m), 1.61-1.66 (4H, m), 2.30 (2H, t, $J = 7.4$ Hz), 4.06 (2H, t, $J = 6.6$ Hz),

^{13}C NMR (CDCl_3): δ 24.9, 25.9, 28.7, 34.5, 64.5, 173.9

The unique signals at 4.23 (t, OCH_2) and 2.64 (t, C(O)CH_2) were assigned to the protons of ϵ -caprolactone. Signals at 4.06 (t, OCH_2), 3.64 (t, HOCH_2), and 2.30 (t, C(O)CH_2) appeared after the onset of polymerization reactions and were assigned to poly(ϵ -caprolactone). The relative intensity of signals at 4.06 to 4.23 and 4.06 to 3.64 were used to calculate monomer conversion and M_n , respectively.

Poly(ω -pentadecalactone)

Synthesis was by Method B and the monomer was ω -pentadecalactone (1 g, 4.16 mmol).

^1H NMR (CDCl_3): δ 1.24-1.63 (24H, m), 2.28 (2H, t, $J = 7.5$ Hz), 4.06 (2H, t, $J = 6.7$ Hz),

^{13}C NMR (CDCl_3): δ 25.4, 26.3, 29.1, 29.6, 29.6, 29.7, 29.9, 29.9, 30.0, 30.0, 30.1, 34.8, 64.8, 174.3

5. Thermal Properties

Polymers were dissolved in CHCl_3 , precipitated in methanol and dried in vacuum before analysis by differential scanning calorimetry (DSC). Summarized data for polymerization products are given in Table S1.

Table S1. Thermal properties of polyesters.

Polymer	$T_g^{[a]}$ [°C]	$T_m^{[b]}$ [°C]	$T_m^{[c]}$ [°C]	$\Delta H_m^{[d]}$ [Jg ⁻¹]	Scan rate [°C min ⁻¹]	Range [°C]
Poly(butylene adipate)	-60.1	56.7	52.3	48.2	5	-85 to +80
Poly(hexylene adipate)	n.d. ^[e]	60.0	-	74.4	5	-85 to +80
Poly(octylene adipate)	n.d. ^[e]	68.6	-	88.4	5	-85 to +80
Poly(cyclohexane dimethylene adipate)	-23.4	107.4	100.1	53.2	5	-85 to +120
Poly(cyclohexane dimethylene suberate)	-35.1	82.6	-	42.1	5	-85 to +100
Poly(cyclohexane dimethylene sebacate)	-40.7	62.1	55.7	36.5	5	-85 to +80
Poly(ϵ -caprolactone)	n.d. ^[e]	56.1	-	58.6	10	-85 to +80
Poly(ω -pentadecalactone)	n.d. ^[e]	96.1	-	125.6	10	-85 to +120

[a] glass transition temperature; [b] peak melting temperature of the higher melting transition; [c] peak melting temperature of the lower melting transition; [d] heat of fusion; [e] n.d.=not determined.