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Lipase-catalyzed synthesis of aliphatic polyesters via copolymerization of lactide with diesters and diols

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ABSTRACT

Aliphatic lactate-bearing copolyesters were successfully synthesized via copolymerization of L-lactide (LLA) with diesters and diols using *Candida antarctica* lipase B (CALB) as the catalyst. The resultant copolymers had a M_w up to 38,000 Da with M_w/M_n between 1.5 and 2.0, and contained L-lactate units (up to 53 mol%), C_6–C_{12} diester units, and C_4–C_6 alkylene units in the polymer chains. The lactate repeat units were present primarily as lactate–lactate diads in the polymers. The LLA-diester-diol copolymers were purified in good yield (70–85%) and all purified copolymers were optically active. Hydrolytic degradation study shows that LLA-diethyl adipate-1,6-hexanediol (LLA-DEA-HD) copolymers are degradable polymers as the molecular weight (M_w) of the copolymer with 53% lactate units decreased by ~70% upon incubation in PBS solution under physiological conditions (37 °C, pH of 7.4) for 80 days. The LLA-diester-diol copolymers are thermally stable up to at least 300 °C with the temperature of maximum degradation rate ranging from 380 to 410 °C. The copolymers exhibit a wide range of physical properties (e.g., from white solid to wax and liquid) depending on their structure and composition. In particular, the LLA-DEA-HD and LLA-DEA-1,4-butanediol copolymers with ~50 mol% lactate units are colorless, viscous liquids at ambient temperature. Biodegradable liquid polymers are potentially useful biomaterials for drug delivery to treat ocular ailments because of their good compatibility with sensitive soft tissues.

1. Introduction

Lactate-containing polyesters are a family of biodegradable materials with important biomedical applications, which typically consist of lactate units or a mixture of lactate and lactone (e.g., glycolide, ε-caprolactone) units in the polymer chains [1–3]. In particular, poly(L-lactide) (PLLA), poly(D,L-lactide) (PDLLA), and poly(L-lactide-*co*-glycolide) (PLGA) have been widely used as raw materials to construct various medical devices including sutures, screws, meniscus staples, tacks, plates, meshes, and spinal cages for clinic applications. These polymeric materials are currently produced via ring-opening polymerization of lactides or ring-opening copolymerization of lactides with lactones using an organometallic catalyst [4–6]. However, in these processes, the metal contents in the polymer products need to be minimized in order to meet the requirement for the polymers to serve as medical grade materials.

In the past two decades, enzymes (particularly lipases) have been successfully employed for synthesis of polyesters, polycarbonates, and poly(carbonate-*co*-esters) with a wide variety of molecular structures [7–11]. Comprehensive reviews are available on enzyme-catalyzed ring-opening polymerization of lactones [12–17] and cyclic carbonates [18–20], polycondensation between diacids (or diesters) and diols [21–23], polymerization of hydroxy acids [21,24], polycondensation of organic carbonates with diols [25–27] or organic carbonates with diesters and diols [28,29], copolymerization of lactone with diester and diol [30–33], and copolymerization of lactone with organic carbonate and diol [34,35]. In contrast to chemical processes, the enzymatic synthesis methods have several distinct advantages that include mild reaction conditions, high tolerance of functional groups, higher catalyst selectivity, and resultant high purity of products that are also metal-free.

Despite the large number of enzymatic polymers disclosed in literature, only limited number of reports were found on synthesis of polylactides and copolymers bearing lactate units. It is particularly challenging to incorporate polar, short chain monomers into copolymer chains [36,37]. Enzymatic polylactides were first prepared via ring-opening polymerization of lactides using lipase PS, porcine pancreatic lipase, and *Candida cylindracea* lipase as catalysts (3–29% polymer yield after 7 days) [38,39].

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Addition of ethylene glycol as an initiator was found to promote poly(L-lactide) synthesis [40]. When polyols were employed as initiators, branched polylactides were formed [41]. Syntheses of poly(L-lactide-*co*-glycolide) and poly(DL-lactide-*co*-glycolide) were disclosed using lipase PS as the catalyst (no yield reported) [42]. Copolymerization of lactides with trimethylene carbonate in the presence of porcine pancreatic lipase generated poly(lactide-*co*-trimethylene carbonates) in 6–40% yield after 7 days [43]. *Candida antarctica* lipase B (CALB) has also been evaluated as a catalyst for lactide polymerization. At mild reaction conditions (50–70 °C), CALB was an active catalyst for polymerization of D-lactide, but not L-lactide [44]. Oligomers were formed during CALB-catalyzed copolymerization of ε–caprolactone with DL-lactide [45].

In this work, lipase-mediated synthesis of aliphatic copolyesters bearing lactate units is achieved via copolymerization of lactide with diesters and diols. The polymer molecular weights were measured by gel permeation chromatography (GPC), and the polymer structures were characterized by ^{1}H and ^{13}C NMR spectroscopy. The thermal properties of the copolymers were analyzed by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The hydrolytic degradation rate of a representative L-lactide-diester-diol copolymer was also investigated. To the best of our knowledge, enzymatic synthesis of copolyesters comprising of lactate, diester, and alkylene units via copolymerization of lactide with diesters and diols has not been reported previously. In addition, this synthesis method allows preparation of a number of biodegradable polyesters from various renewable monomers including L-lactide, adipates or succinates, 1,4-butanediol, and 1,6-hexanediol [46].

2. Experimental section

2.1. Materials

Diethyl adipate (DEA, 99%), diethyl dodecanedioate (DED, 98%), 1,6-hexanediol (HD, 99%), 1,4-butanediol (BD, 99%), L-lactide (LLA, 98%), and diphenyl ether (99%) were purchased from Aldrich Chemical Co. and were used as received. Phosphate buffered saline (PBS) solution (pH = 7.4) was purchased from Invitrogen. Immobilized *Candida antarctica* lipase B (CALB) supported on acrylic resin or Novozym 435, chloroform (HPLC grade), dichloromethane (99+%), hexane (97+%), and chloroform-*d* were also obtained from Aldrich Chemical Co. The lipase catalyst was dried at 40 °C under 1.0 mmHg for 20 h prior to use.

2.2. Instrumental methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 500 spectrometer. The chemical shifts reported were referenced to internal tetramethylsilane (0.00 ppm) or to the solvent resonance at the appropriate frequency. The number and weight average molecular weights (M_n and M_w, respectively) of polymers were measured by gel permeation chromatography (GPC) using chloroform as the eluent and narrow polydispersity polystyrenes as the standards according to previously reported procedures [23]. Thermogravimetric analysis (TGA) measurements were carried out using a TA-TGA Q50 instrument. The analyses were performed at 10 °C/min from 40 to 550 °C under nitrogen flow. Differential Scanning Calorimetry (DSC) measurements were performed in a nitrogen atmosphere using a TA-DSC Q200 apparatus equipped with a Refrigerated Cooling System (RCS1-1289). DSC scans were run at 20 °C/min from −75 to 150 °C. Controlled cooling at 10 °C/min was applied between scans. Melting temperature (T_m) was taken at the peak maximum of endotherm. In the presence of multiple peaks, the temperature of the main peak was taken as T_m. Optical rotation values of chiral copolyesters were measured in dichloromethane solution at 20 °C and 589 nm (the sodium D line) using a Perkin Elmer Polarimeter 341.

2.3. General procedure for lipase-catalyzed copolymerization of lactide with diesters and diols

The ring-opening and condensation copolymerizations were performed in diphenyl ether solution using a parallel synthesizer connected to a vacuum line with the vacuum (±0.2 mmHg) controlled by a digital vacuum regulator. In a typical experiment, monomers (lactide, diester, and diol), Novozym 435 catalyst (10 wt % vs. total monomer), and diphenyl ether solvent (200 wt% vs. total monomer) were blended to form reaction mixtures. The copolymerization reactions at various temperatures were performed in two stages: first stage oligomerization under 1 atm pressure of nitrogen gas for 16–20 h, followed by second stage polymerization under high vacuum (1–2 mmHg) for up to 72 h. The formed polymer products were analyzed by GPC to measure their molecular weights and by NMR spectroscopy to determine their molecular structures.

2.4. Copolymerization of L-lactide (LLA) with diethyl adipate (DEA) and 1,6-hexanediol (HD)

Reaction mixtures consisting of monomers (LLA, DEA, and HD), Novozym 435 catalyst (10 wt% vs. total monomer) and diphenyl ether solvent (200 wt% vs. total monomer) were magnetically stirred at preset temperatures under 1 atm pressure of nitrogen gas for 16–20 h. Subsequently, the reaction pressure was reduced to 1–3 mmHg and the reactions were allowed to take place at same temperatures for additional 3 days. Three sets of experiments were performed to study the temperature effects and feed composition effects on the copolymerization reaction, and to investigate the polymer chain growth during the polymerization.

To assess temperature effects on the copolymerization, the monomer molar ratio was fixed at 1:2:2 LLA/DEA/HD and the copolymerization reaction was carried out at 50, 60, 70, 80, and 90 °C, respectively. The first-stage reaction was run under 1 atm pressure of nitrogen gas for 19 h and the second-stage reaction was performed under 1.5 mmHg vacuum for 72 h. To assess monomer feed ratio effects on the copolymerization, different LLA/DEA/HD monomer feed ratios (1:8:8, 1:3.72:3.72, 1:1.08:1.08, 1:0.5:0.5) were used. The two-stage polymerization reactions were carried out at 90 °C under similar conditions as described above (first stage: 1 atm nitrogen, 19 h; second stage: 1.5 mmHg, 72 h). At the end of the two sets of polymerization reactions, aliquots were withdrawn from the resultant product mixtures for polymer molecular weight and structural analysis. To analyze the polymer molecular weights, the aliquot samples were dissolved in HPLC-grade chloroform and filtered to remove the enzyme catalyst. Polymers were not fractionated by precipitation prior to analysis of molecular weight and structure. The filtrates containing whole products were analyzed by GPC using polystyrene standards to measure polymer molecular weights. To determine polymer structures, the aliquots were dissolved in chloroform-*d*. The resultant solutions were filtered to remove catalyst particles and then analyzed by ^{1}H and ^{13}C NMR spectroscopy.

To study the polymer chain growth during the copolymerization, two copolymerization reactions using 1:8:8 and 1:2:2 LLA/DEA/HD molar ratios were performed. Both reactions were carried out under same conditions: 90 °C, 1 atm nitrogen, 18 h