Colloids and Surfaces B: Biointerfaces 113 (2014) 254–260

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Toward one-pot lipase-catalyzed synthesis of poly(ε-caprolactone) particles in aqueous dispersion

Pranee Inprakhon[a,b,*], Pabhangkon Panlawan[a], Thunyarat Pongtharankul[a], Emmanuelle Marie[c,d,1], Lars O. Wiemann[b], Alain Durand[c,d], Volker Sieber[b]

[a] Department of Biotechnology, Faculty of Science, Mahidol University, Rama VI Road, Phayathai, Bangkok 10400, Thailand
[b] Project Group BioCat, Fraunhofer Institute for Interfacial Engineering and Biotechnology, Schulgasse 11a, 94315 Straubing, Germany
[c] CNRS, LCPM, FRE 3564, Nancy, F-54001, France
[d] Université de Lorraine, LCPM, FRE 3564, Nancy, F-54001, France

ARTICLE INFO

Article history:
Received 21 July 2013
Received in revised form 4 September 2013
Accepted 5 September 2013
Available online 16 September 2013

Keywords:
Lipase
ε-caprolactone
Ring-opening polymerization
Aqueous dispersion

ABSTRACT

The preparation of polyester particles using enzyme-catalyzed (lipase from *Candida antarctica B*, CALB) ring-opening polymerization of ε-caprolactone (ε-CL) in aqueous dispersion was demonstrated for the first time. Immobilization of CALB enabled a significant increase of the number–average degree of polymerization of ε-CL oligomers (up to 38) as compared to dissolved CALB (8 at the maximum). The nature and amount of lipase, as well as the nature of the support material were identified as key parameters controlling ring-opening polymerization of ε-CL in aqueous dispersion. In addition, the involvement of solubilized monomers in polymerization elementary reactions was demonstrated and the consequences on oligomers average length were detailed. An overall mechanism of lipase-catalyzed ε-CL polymerization in aqueous dispersion taking into account the colloidal nature of reaction medium was proposed on the basis of experimental results.

1. Introduction

Polylactones in general and poly(ε-caprolactone) (PCL) for instance find numerous applications in the medical field either under the form of bulk materials or as particles [1]. For a long time, there has been a significant interest for the development of synthetic pathways for preparing PCL with a reduced use of toxic products (reactants, catalysts, and solvents for instance). Enzymes like lipases (EC 3.1.1.3) were used to catalyze ring-opening polymerization (ROP) of lactones in organic solvent or in bulk [2]. Despite the advantages provided by lipases, some questions remained like the relatively slow reaction rates, the need to control water activity and multi-step work-up required to obtain PCL particles. For the latter, lipase-catalyzed ROP of lactones in aqueous dispersion had been proposed recently as a greener process avoiding the use of organic solvents, maintaining high enzymatic activity at water/lactone interface and providing a one-pot synthesis of polyester particles. A few reports appeared about lipase-catalyzed ROP in aqueous dispersions [3–7]. However, until now, the enzymatic polymerization in aqueous dispersion was reported only for very hydrophobic monomers (16-membered lactone, ω-PDL and 12-membered lactone, UDL). More hydrophilic lactones like lactide and ε-caprolactone (ε-CL) did not polymerize under these conditions. If we consider the particular case of ε-CL, its ROP in aqueous dispersion has never been reported except recently using metal triflates as catalysts [8].

In this work, we reported for the first time lipase-catalyzed ROP of ε-CL in aqueous dispersion. The molar mass distribution of PCL was related to colloidal properties of heterogeneous reaction medium. The results were compared to those obtained with more hydrophobic lactones. Finally, a mechanism was proposed to account for the specific case of a polar monomer like ε-CL.

2. Experimental

2.1. Materials

Monomers (ε-caprolactone (ε-CL), oxacyclododecan-2-one (UDL) and 15-pentadecalactone (ω-PDL)), lyophilized lipases (*Candida antarctica* B or CALB, recombinant from *Aspergillus oryzae* with 9 U mg^{-1}, and *Burkhodoria cepacia* or PS with 32,200 U g^{-1}), immobilized CALB (N435 or CALB immobilized on acrylic resin Lewatit VP OC1600 with ≥10,000 U g^{-1} from Novozyme A/S), immobilized PS (IM or PS immobilized on diatomaceous earth with >500 U g^{-1}

* Corresponding author at: Project Group BioCat, Fraunhofer IGB, Schulgasse 11a, 94315 Straubing, Germany. Tel.: +49 9421 187350; fax: +49 9421 187360.
E-mail address: pranee.inprakhon@igb.fraunhofer.de (P. Inprakhon).
[1] Permanent address: ENS-Département de Chimie, UMR 8640 CNRS-ENS-UPMC, 24 Rue Lhomond, 75005, Paris, France.

0927-7765/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.09.011

Table 1
ROP of ε-CL, UDL and ω-PDL in aqueous dispersion catalyzed by lipases. Reaction conditions: 0.2 mole of lactones dispersed in 1 L of water at 60 °C for 72 h.

Lipase	Activity (U mL^{-1})	ω-PDL		UDL		ε-CL	
		Yield (%)	$\overline{DP_n}$	Yield (%)	$\overline{DP_n}$	Yield (%)	$\overline{DP_n}$
PS	322	60	4	34	10	–	–
CALB	45	81	4	N/D[a]	N/D[a]	89	5
Control without enzyme	–	–	–	N/D[a]	N/D[a]	–	–

[a] N/D: not identified due to the high price of UDL. We assumed that the $\overline{DP_n}$ should follow the same trend as PS based on literature [7].

and Sol-Gel-AK or PS immobilized in Sol–Gel with >40 U g^{-1}) were purchased from Sigma Aldrich and used as received. Chirazyme L2 (L2 or CALB immobilized on a macroporous phenolic type carrier with 200 U g^{-1}) was a generous gift from Boehringer Mannheim, Germany.

2.2. Methods

2.2.1. Synthesis of oligolactones in aqueous medium

The lipases-catalyzed ROP of lactones in aqueous medium was carried out as described previously [7]. The reaction mixture (2.0 mmol of monomers, 10 mL of distilled water and 50 mg of CALB or 100 mg of PS) was stirred vigorously at 60 °C for 72 h. Control ROP reactions followed exactly the same procedure but without lipases. Lactones were recovered unchanged after those experiments.

The fractionation of oligo(CL) was carried out by dissolving the obtained oligo(CL) in 1 mL of chloroform, followed by pouring into 100 mL of cold methanol to separate high molar mass oligomers which precipitated, named oligo(CL)$_{prep}$, from low molar mass oligomers which remained solubilized in supernatant, named oligo(CL)$_{sol}$. Both fractions of oligo(CL)s were dried in vacuo at 45 °C.

2.2.2. Determination of ring-opening activity of lipases

The ring-opening (RO) assay conditions were optimized using the method of Saktaweewong et al. [9]. The ring-opening activities of L2 (0.6 mg mL^{-1}) and CALB (0.12 mg mL^{-1}) were determined by pH-stat using 25 mL of 100 mM ε-CL as reaction medium. All experiments were duplicated. One enzyme unit (U) hydrolyze (ring-opening) of 1 μeq. of hydroxyhexanoic acid from ε-CL in 1 min at 25 °C (pH 7).

2.2.3. Stability of L2 and CALB under ROP conditions

L2 (200 mg) was suspended in 20 mL of water and stirred vigorously at 60 °C for a specific period, filtered, washed several times with double distilled water and dried by lyophilization before measuring RO activity in the presence of emulsified ε-CL as described in Section 2.2.2. For CALB, 3 mg of CALB dissolved in 25 mL of water were incubated at 60 °C for a specific period prior to measuring the RO activity as described for L2.

2.2.4. Ring-opening polymerization kinetics

ROP of ε-CL was carried out in four screw-cap bottles containing the same mixture (228.25 mg of ε-CL, 10 mL of distilled water and 100 mg of L2) as described in Section 2.2.1 for 0.25, 1, 24, and 72 h. The separation of products and fractionation were performed as previously described in Section 2.2.1. Each reaction/fractionation experiment was done in triplicate.

2.2.5. Enzymatic degradation of oligo(CL) in aqueous medium

The degradation of oligo(CL)$_{prep}$ having a number–average molar mass equal to 3600 g mol^{-1} was carried out following the procedure described in Section 2.2.1 during 1, 4 and 6 h. The recovered polymers were then analyzed by size exclusion chromatography. Control hydrolytic reactions were conducted during 6 h with the same oligo(CL)$_{prep}$ but without lipases. The oligo(CL)$_{sol}$ fractionated from reaction medium after 72 h of ROP of ε-CL was used as a control of non-hydrolyzed oligo(CL)$_{sol}$. In an attempt to reach complete hydrolysis, experiments with oligo(CL)$_{sol}$ ($\overline{M_n}$ = 860 g mol^{-1}) and oligo(CL)$_{prep}$ ($\overline{M_n}$ = 2700 g mol^{-1}) were left proceed during 72 h. In both cases, the complete hydrolysis was ascertained by ^{1}H-NMR analysis.

2.2.6. Characterization of oligo(CL) products and reaction medium

The ^{1}H-NMR spectra of oligo(CL) were recorded in $CDCl_3$ using a BRUKER 300 MHz spectrometer.

Molar mass distributions of oligo(CL) were characterized using size exclusion chromatography (SEC) device (Waters, Model 410) equipped with a differential refractometer and a multi-angle laser light scattering detector at room temperature. Tetrahydrofuran was used as eluent with a flow rate of 0.5 mL min^{-1}. Three different PL gel columns (50, 100, 500 Å) were used and $\overline{M_n}$ as well as weight-average molar masses ($\overline{M_w}$) were calculated using signals from both detectors. Particle size distributions of oligo(CL) and L2 dispersions were characterized by laser granulometry (Mastersizer 2000, Malvern Instrument Co., Ltd.) using a refractive index of 1.463. Thermogravimetric analysis was performed using PERKIN-ELMER DSC7. The water content (wt%) of saturated ε-CL was measured using Mettler-Toledo C20 system.

3. Results and discussion

3.1. Synthesis of oligo(ε-caprolactone) in aqueous dispersion

Lyophilized lipases, PS and CALB, already reported as active for ROP of ε-CL, UDL and ω-PDL in organic solvents as well as in bulk were tested for their catalytic activities toward ROP in aqueous dispersion (Table 1) [3,10,11].

PS and CALB catalyzed ROP in aqueous dispersion for the more hydrophobic lactones, ω-PDL and UDL, a result that was in accordance with previous studies [3,4,7]. Nevertheless, the number–average degree of polymerization, $\overline{DP_n}$, remained lower than 10. Contrary to what had been reported in bulk, PS did not catalyze ROP of ε-CL in aqueous dispersion [10]. This can be related to the catalytic activity of PS. Indeed, this lipase requires to be adsorbed onto a hydrophobic surface to catalyze ROP in biphasic medium [7]. Since ε-CL has a significantly higher polarity than other lactones, as indicated by its dipole moment [12], adsorption of lipase PS may have been limited and polymerization could not occur to a significant extent [13]. In order to support that assumption, we determined Michaelis constants ($K_{m,app}$) characterizing PS-catalyzed ring-opening of ε-CL and ω-PDL (by titration of formed carboxylic acids). $K_{m,app}$ was 22-fold higher for ε-CL than for ω-PDL (Supplementary material Fig. S1 and Table S1).

Contrary to PS, CALB catalyzed oligomerization of ε-CL in aqueous dispersion. To the best of our knowledge, this was the first report of ROP of ε-CL in aqueous environment. Our result was different from that reported by other authors who also investigated CALB activity on ε-CL [4]. The discrepancy might be explained by the low weight ratio of CALB to ε-CL used by these authors (0.15 wt%), which was approximately 146 times lower than that used the