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Fusarium solani pisi cutinase-catalyzed synthesis of polyamides

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ABSTRACT

Polyamides, or nylon, are widely used in fiber and engineering plastic materials, due to their good mechanical and thermal properties. Synthesis of oligomers from nylon-4,10, nylon-6,10, and nylon-8,10 were performed via polycondensation of diamines (1,4-butanediamine, 1,6-hexanediamine, and 1,8-diaminooctane) and diester (diethyl sebacate). These reactions were catalyzed by immobilized cutinase from *Fusarium solani pisi* on Lewatit beads, cutinase in the form of cross-linked enzyme aggregates (CLEA), or by immobilized Lipase B from *Candida antarctica* (N435). The highest maximal degree of polymerization (DP_{max}), up to 16, can be achieved in the synthesis of nylon-8,10 catalyzed by CLEA cutinase in diphenyl ether at 70 °C. By performing a reaction at cutinase optimal temperature (70 °C), CLEA cutinase in the synthesis of nylons shows good catalytic activity, like N435.

1. Introduction

Polyamides, also known as nylon, are widely used in fiber and engineering plastic materials, due to their good mechanical and thermal properties [1]. Synthesis of nylons can be accomplished via ring opening polymerization [2] and polycondensation [3]. Industrial synthesis of nylon is conducted at high temperatures, which leads to thermal degradation and results in undesired polymer products [4]. On the other hand, alternative options for synthesizing polymers at relative low temperatures can be achieved via enzymatic polymerization. Enzymatic polymerizations are known as environmentally friendly reactions, as they can be carried out in relatively low temperatures, and they use enzymes as catalysts [5–10].

Candida antarctica lipase B CALB is a highly used enzyme in biocatalysis. CALB is commercially available as N435, which is physically immobilized CALB within macroporous poly(methyl methacrylate) resin (known as Lewatit OC VOC 1600) [11]. N435 is known to have high catalytic activity over a broad range of esters, amides, and thiols [12]. CALB has been used in esterification and transesterification [13], aminolysis [14,15], and synthesis of polyester [5,7,16], polyamides [17–19] and poly(amide-co-ester) [20].

Here, we present, for the first time, synthesis of polyamides catalyzed by *Fusarium solani pisi* cutinase. Cutinases are hydrolytic enzymes that degrade cutin, which contains polyester and epoxy fatty acids (C_{16} and n-C_{18}). This enzyme is known as a small carboxylic ester hydrolase (M_w 22 kD) that bridges functional properties between lipases and esterases [21]. Like CALB, *F. solani pisi* cutinase belongs to the α/β hydrolase family, which has a catalytic triad in Ser120, His188, and Asp175. It is known, that the active site of this enzyme is not covered by a lid [21,22] and therefore *F. solani pisi* cutinases do not exhibit interfacial activation, just as CALB [23].

In order to increase temperature resistance and tolerance toward organic solvents [24] *F. solani pisi* cutinase was immobilized on Lewatit beads or used as cross-linked enzyme aggregates (CLEA). Using an immobilized enzyme allows it to be separated from the product more easily than a free enzyme, thereby minimizing protein contamination of the product [25,26].

The enzymatic polymerizations of diethyl sebacate and aliphatic diamines (C_4, C_6, and C_8) catalyzed by immobilized *F. solani pisi* cutinase on Lewatit beads or as CLEA cutinase are presented. The general reaction scheme is shown in Scheme 1. The same reaction was

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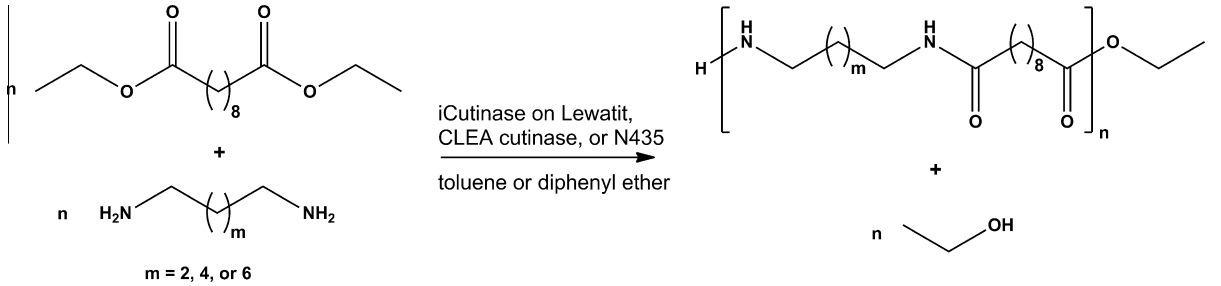

Scheme 1. Polycondensation of diethyl sebacate and diamines catalyzed by icutinase on Lewatit, CLEA cutinase, or N435.

also performed using N435 to determine whether cutinase displays catalytic activity in the synthesis of polyamides, as N435 does.

2. Experimental

2.1. Materials

F. solani pisi cutinase (Novozym 51032) was a gift from Novozymes, Denmark. Lewatit OC VOC 1600 was donated by Lanxess, Belgium. Ethanol, formic acid, and calcium hydride were purchased from Merck. 1,2-Dimethoxyethane, sodium phosphate dibasic, sodium phosphate monobasic, 4-nitrophenol butyrate, sodium cholate hydrate, ε-caprolactone, chloroform-*d*, diethyl sebacate, hexafluoroisopropanol, sodium trifluoroacetate, Lipase acrylic resin from *C. antarctica* (N435), molecular sieves 4 Å, and trifluoroacetic acid-*d* (TFA-*d*) were purchased from Sigma–Aldrich. A BCA Protein assay kit was purchased from Thermo Scientific. 1,8-Diaminooctane, 4-nitrophenol, and 2-(4-hydroxy-phenylazo)benzoic acid (HABA) were purchased from Fluka. 1,4-Butanediamine, 1,6-hexanediamine, tetrahydrofuran, and glutaraldehyde were obtained from Acros. Except for toluene, diethyl sebacate, and diamines, all chemicals were used without further purification. Toluene was dried by a solvent purification system (SPS). Diethyl sebacate (DES) was dried using CaH_2 and vacuum distilled. 1,4-Butanediamine (BD), 1,6-hexanediamine (HD), and 1,8-diaminooctane (DAO) were purified by sublimation.

2.2. Immobilization of F. solani pisi on Lewatit

The Lewatit beads were first activated with ethanol and dried under vacuum for 60 min to remove traces of ethanol [27]. Then, beads (0.2 g) were added to 3 mL cutinase (used as received with concentration of 20 mg/mL). The samples were incubated in a shaker at 100 rpm at 4 °C for 48 h. Subsequently, the supernatant was removed, and the remaining beads were washed with sodium buffer phosphate (0.1 M, pH 7.8). The concentration of cutinase that remained in the supernatant and washing solutions was determined using the BCA assay kit. The amount of immobilized cutinase on the Lewatit was determined as enzyme loading. The immobilized cutinase on Lewatit was freeze-dried for 48 h.

2.3. Preparation of CLEA F. solani pisi cutinase

Three milliliters of cutinase stock solution (20 mg/mL) were dissolved in 6 mL sodium phosphate buffer (100 mM, pH 7) [28]. Subsequently, 18 mL of 1,2-dimethoxylethane, and 480 μL glutaraldehyde (25% w/v in water) were added. The mixture was stirred at 4 °C for 17 h. After 17 h, 6 mL of 1,2-dimethoxyethane was added. Then, the mixture was centrifuged for 20 min at 7000 rpm. After centrifugation, the supernatant was separated from the residue. Later, the residue was washed with 30 mL of 1,2-dimethoxyethane, centrifuged, and decanted. The washing step was repeated three times. CLEA was collected and dried in a vacuum oven at 25 °C for 1 h.

2.4. Cutinase hydrolysis assay

The cutinase hydrolysis activity was determined by hydrolysis of 4-nitrophenyl butyrate (p-NPB) to 4-nitrophenol (p-NP). iCutinase on Lewatit or CLEA cutinase (10 mg) were added to 1 mL of sodium phosphate (11.3 mM), tetrahydrofuran (0.43 M), and pNPB (0.55 mM) in sodium phosphate buffer (50 mM, pH 7.0). The hydrolytic activities of cutinase were measured by UV–Vis spectrophotometer at 399 nm in a time range of 1 min against the blank solution [29]. Molar absorptivity of p-NP was considered to be 7919.2 M^{-1} cm^{-1} from the calibration curve. Specific activity of cutinase was defined as nmol of p-nitrophenol for 1 min per gram solid support.

2.5. Cutinase synthetic assay

The cutinase synthetic activities were determined by ring opening polymerization of ε-caprolactone. One milliliter of ε-caprolactone was added to a 25 mL two-neck flask containing of immobilized cutinase (10 mg or 10 μL for free cutinase). The reactions were carried out at 70 °C for 24 h at 100 rpm, under N_2 atmosphere. The monomer conversion and the degree of polymerization were determined by ^1H NMR measurements using $CDCl_3$ as solvent.

2.6. General procedures of enzymatic synthesis of nylon-4,10, nylon-6,10, and nylon-8,10

Nylons were synthesized via two different reaction conditions—one-step and two-step reactions.