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Immobilized lipase on porous silica particles: Preparation and application for biodegradable polymer syntheses in ionic liquid at higher temperature

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ABSTRACT

Porous silica particles (PSP) modified with different surface active groups were prepared for covalent immobilization of porcine pancreas lipase (PPL). Organosilanes combined with reactive end amino-group or epoxy-group were employed for the modification through silanization process. Polyethylenimine and long chain alkyl silane coupling agent were also used in the modification process. Several modification-immobilization strategies were performed, while good coupling yield could be achieved within the range of 86.2–158.2 mg of native PPL per gram of the carrier. Furthermore, at higher temperature, the resulting immobilized PPL (IPPL) could successfully perform the syntheses of polycaprolactone (PCL) and poly(5,5-dimethyl-1,3-dioxan-2-one) (PDTC) in ionic liquid medium. No polymers could be obtained catalyzed by native PPL, suggesting that IPPL showed much higher catalytic activity than native PPL. Effect of different treatments on the activity of IPPL also showed the long time high temperature stability in ionic liquid medium, contributing to a good combination of immobilization and ionic liquids effect. The catalytic activity of IPPL for polymerization was closely related to both the properties of immobilized enzyme and cyclic monomer. This work would be expected to highlight further careful design of immobilized enzyme for a wide range of application, especially in biodegradable polymers syntheses.

1. Introduction

Enzyme immobilization is the most important route that can provide enzyme many favorable advantages such as increased enzyme activity, increased enantioselectivity, improved stability and recyclability [1]. There are several immobilization strategies including physical adsorption, covalent attachment and entrapment in inorganic and organic matrices. Among them, covalent attachment of enzymes on chemically modified surfaces of solid supports has been intensively investigated, especially the covalent immobilization though silane coupling agents [2–5]. The activity and stability of the immobilized enzyme are closely related to the properties of supports, such as pore size, chemical durability, capacity for ligand binding, *etc.* [6].

Porcine pancreas lipase (PPL) is one of the most widely lipases and is cheaper compared to other commercial microbial and animal lipases [7]. PPL has high stability and activity in anhydrous media as demonstrated in esterification and transesterification reactions. Immobilization techniques could further improve PPL's catalytic activity and stability properties.

Porous silica particles (PSP), activated by methanesulfonic acid [8] and then modified through silanization process with (3-aminopropyl)triethoxysilane (APTES), are highly effective and economic inorganic carriers for enzyme immobilization. Different kinds of enzyme such as papain, acylase and lipase have been successfully immobilized on PSP by covalent method. The resulting immobilized enzyme show thermal activation and good thermal stability [9,10], which permitted potential applications in some novel reaction systems, such as polymerization at higher temperature. As a new environmental friendly method of polymer synthesis, research on enzymatic polymerization has received increasing attentions. Actually and attractively, the covalent immobilized porcine pancreas lipase (IPPL) has been employed successfully for the syntheses of biodegradable polymers [10–15] in bulk condition. For example, poly(trimethylene carbonate) could be effectively synthesized in bulk by IPPL at 100 °C, even after recycling IPPL up to seven times [16]. The good stability, remarkable biosafety as well as comparable activity would ensure enzymatic polymerization as one of the powerful candidates for polymer synthesis.

Polyesters and polycarbonates are two main kinds of biodegradable and biocompatible polymers which have been extensively researched for medical applications. Their most common and effective synthetic method is ring-opening polymerization. For example, poly(ε-caprolactone) (PCL) is usually synthesized by ring-opening polymerization of seven-numbered lactone ε-caprolactone (ε-CL)

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[17,18]. Biodegradable polycarbonates are also generally prepared from six-numbered cyclic carbonates with or without functional pendant groups. However, it was reported that native PPL showed rather low catalytic activity toward the polymerization of ε-CL [19]. Although the covalent immobilized enzyme IPPL showed a relative better activity, no PCL could be obtained at a reaction temperature lower than 140 °C for 240 h. Even at a higher temperature of 180 °C, PCL could only be synthesized with the reaction time more than 144 h [20]. Very interestingly, IPPL showed much better catalytic activity for ring-opening polymerization of cyclic carbonates in bulk condition, which indicated some selectivity of IPPL for different cyclic monomers.

On the other hand, as green solvents, ionic liquids (ILs) have been widely studied due to their low volatility, chemical stability as well as good solubility of many organic or inorganic compounds. Several advantages of ILs as solvents for polymerization such as better control in atom transfer radical polymerization have been also pointed out [21,22]; however, ILs applications in polymer synthesis is still limited compared with their uses in organic chemistry. It is therefore interesting to study and develop new polymerization systems in ILs.

In fact, some researchers have presented that enzymes show both excellent operational and thermal stability in anhydrous ILs owing to their conformational rigidity in dehydrate and high viscous state [23]. Thus enzymatic processes in ILs could be conducted at high temperature if needed. To date, lipase-catalyzed reactions have been carried out in ILs, including esterification, transesterification, alcoholysis, aminolysis, hydrolysis and polycondensations [24].

In this study, the combination of covalent immobilization method, ILs and IPPL-catalyzed ring-opening polymerization was expected to become a versatile and attractive polymerization processes. After activated by methanesulfonic acid, PSP were modified through silanization process with different silane coupling agents, such as (3-aminopropyl)triethoxysilane (APTES) and (γ-glycidoxypropyl)trimethoxysilane (GPTMS). Furthermore, (n-dodecyl)trimethoxysilane (DTMS) and low molecular weight polyethylenimine (PEI, M_n = 423) were also employed to modify the surface properties of PSP. Then, PPL was immobilized on the modified PSP. The comparison between different chemically immobilized enzymes was done in terms of functional group, enzyme loading and catalytic performance using a standard hydrolytic assay (hydrolysis of olive oil). Last but not least, the immobilized enzymes were employed for the first time to perform the ring-opening polymerization in ionic liquid, 1-butyl-3-methylimidazolium hexaflurophospate (abbreviated as $BMIMPF_6$). ε-CL as well as 5,5-dimethyl-1,3-dioxan-2-one (DTC), two kinds of cyclic monomers with quite different polymerizability upon IPPL catalysts in bulk and organic solutions, were also first used as model monomers. IPPL had much better stability and higher retained activities with respect to temperature stability, than the native lipase. To the best of our knowledge, the strategies of using different modification/coupling methods aiming to immobilize lipase on the particles, and also catalyzing polymerization in ionic liquids, have not been reported in the literature yet. The careful design of immobilization and polymerization techniques would be good for polymer syntheses.

2. Materials and methods

2.1. Materials

Porcine pancreas lipase (PPL) was purchased from Sigma Co. with an activity of 179.5 U/mg protein (at pH 6.0, using olive oil as substrate) and used as received. The purity of the enzyme was confirmed by a single band of molecular weight 55 kDa observed in the SDS-PAGE. Porous silica particle (PSP, 200–300 mesh) was obtained from QingDao Haiyang Chemical Co. of China. (3-aminopropyl) triethoxy silane (APTES), (n-dodecyl)trimethoxysilane (DTMS) and (γ-glycidoxypropyl) trimethoxysilane (GPTMS) were all obtained from the Chemical Plant of Wuhan University of China and redistilled before use. Polyethylenimine (PEI, M_n = 423) was purchased from Aldrich and used without further purification. 5,5-Dimethyl-1,3-dioxan-2-one (DTC) was synthesized according to the reference [25]. ε-CL from Aldrich was redistilled prior to use. 1-Butyl-3-methylimidazolium hexaflurophosphate ($BMIMPF_6$) was purchased from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences. Glutaraldehyde (25%, w/v) and olive oil were purchased from Sinopharm Chemical Reagent Co. of China. All other reagents used in this study were of analytical grade.

2.2. Measurements

^{1}H NMR spectra was recorded on a Varian Mercury-VX 300 apparatus using $CDCl_3$ as the solvent. Gel permeation chromatography (GPC) measurements were detected on Waters-2690D HPLC and a 2410 refractive index detector. Tetrahydrofuran was used as the eluent at a flow rate of 0.3 mL/min. Waters MILLIENIUM^{32} module software was used to calculate molecular weights based on a universal calibration curve generated by narrow molecular weight distribution polystyrene standards.

The primary amine concentration of functional PSP was measured by three methods: the weight increment method (WI), the thermogravimetric analysis (TG) technique as well as ultraviolet spectrophotometric method (UV). The former two results indicated the whole amino group concentration within the carriers, whereas the last result indicated the active amino group with real capability for enzyme binding.

The thermogravimetric analysis (TGA) was registered with a NETZSCH STA 449F3 operator at 10 °C/min heating rate from room temperature to 1200 °C in a N_2 atmosphere.

The ultraviolet spectrometric analysis was performed on Perkin-Elmer Lambda Bio 40 UV-VIS Spectrophotometer. 4-Nitrobenzaldehyde (4-NBA) was used as a chemical tag, which could react specifically with primary amine to form a Schiff base while a reversible reaction could be obtained by hydrolysis [26,27]. A typical procedure was as follows: A weighted functional PSP was washed thoroughly with anhydrous methanol solution, and then mixed at 37 °C for 12 h with anhydrous methanol (100 mL) containing 4-NBA (40 mg) and acetic acid (0.08 mL). After also washed entirely with acetic acid/anhydrous methanol solution, the samples were treated at 30 °C for another 1 h with anhydrous methanol (37.5 mL) containing water (37.5 mL) and acetic acid (0.1 mL). The upper solution was then used to measure the absorption intensity of 4-NBA at 268 nm. The result was expressed as the mean value of three independent measurements.

Enzymatic activity assay was carried out according to the classical olive oil emulsion method [10]. Emulsion of olive oil was produced by emulsifying 25 mL olive oil and 75 mL 2% PVA solution (20 g PVA/1000 mL water) with intensive mixing at 5–10 °C. The emulsion (5 mL) and 4 mL PBS (pH 6.0, 0.1 mol/L) was mixed together and preheated in water bath at 37 °C for 5 min. Then, a weighted IPPL or native lipase was put into the mixture to catalyze the hydrolysis reaction for 30 min, while the reaction was terminated by adding 5.0 mL alcohol–acetone solution (1:1, vol/vol). Blank samples were prepared in parallel with adding the alcohol–acetone solution just after the addition of the enzyme. The fatty acid produced in the reaction was quantified by the volume of the consumed alkali solution. One unit of lipase activity (U) was defined as the amount of enzyme required to produce 1 μmol fatty