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Immobilization of lipase from *Candida rugosa* on electrospun polysulfone nanofibrous membranes by adsorption

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Abstract

Polysulfone composite nanofibrous membranes were prepared by electrospinning and were used to immobilize lipase from *Candida rugosa* by physical adsorption. Field emission scanning electron microscopy was used to evaluate the morphology and diameter of the nanofibers. PVP and PEG were used as additives to render the nanofibrous membranes biocompatibility favored by immobilized enzyme. Effects of post-treatment, additive concentration, pH and temperature were investigated on the adsorption capacity and activity of immobilization preparations, as well as thermal stability. It was found that (1) post-treatment had no significant effect on the adsorption capacity; (2) the increment of PVP or PEG concentration was negative for the adsorption capacity but positive for activity of immobilized lipase; (3) the immobilized lipase showed less sensitivity for pH and higher optimum temperature. Thermal stability for the immobilization preparations was enhanced compared with that for free preparations. The kinetic parameters of the free and immobilized lipases, K_m and V_{max} were also assayed. Results indicated that K_m and V_{max} for the immobilized lipases were higher and lower than those for free lipase, respectively.

Keywords: Electrospinning; Polysulfone; Nanofibrous membrane; Enzyme immobilization; Lipase; Adsorption

1. Introduction

Enzymatic biotransformations have been pursued extensively for a wide range of important chemical processing applications, largely as a result of their unparalleled selectivity and mild reaction conditions [1]. In many cases, however, the low catalytic efficiency and stability of enzymes are considered as barriers for the development of large-scale operations and applications. The performance of immobilized enzyme depends greatly on the characters and structure of the carrier materials. Many efforts have been concentrated on modifying the carriers, in order to make the carriers more suitable for enzyme immobilization and catalysis, such as rendering biocompatibility, hydrophilicity, etc. [2–6]. Nevertheless, even for the modified supports, the enzyme loading is usually considerably low. Alternatively, high enzyme loading can be achieved with porous materials such as membranes, gel matrices, and porous particles [1,7–13]. Recent efforts using nanostructured materials are an intriguing approach since all these materials can provide a large surface area for the attachment of enzymes [14]. Among them, electrospun nanofibers show some attractive prospects, compared to the other nanostructures. On one hand, nanofibers can relieve remarkably diffusion resistance of the substrates/products, due to the shortened path of diffusion resulting from the reduction of the geometric size of the enzyme support, compared to porous materials; on the other hand, the nanofibers, which can be processed into various structures such as non-woven mats, or well-aligned arrays, are more conveniently recovered and more durable than other nanoparticles or carbon nanotubes. Furthermore, electrospinning is simple and versatile. Based on the above advantages, electrospun nanofibers have attracted a great deal of attention as enzyme carriers [1,14–19].

Lipase (triacylglycerol acyl ester hydrolyses, EC 3.1.1.3) is an enzyme possessing an intrinsic capacity to catalyse the cleavage of carboxy ester bonds in tri-, di-, and monoacylglycerols to glycerol and fatty acids [20]. The enzyme is distributed among higher animals, plants and micro-organisms in which it plays a key role in the lipid metabolism. Moreover, a promising property

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of lipases is their activation in the presence of hydrophobic interface [21], and in this case, important conformational rearrangements take place, yielding the "open state" of lipases. Among kinds of materials, polysulfone showed some interests, due to its hydrophobicity, facile process, and chemical inertness. However, the poor biocompatibility of this material may cause nonbiospecific interaction, protein denaturation, and enzyme activity loss. Several approaches have been provoked to modify the materials, such as surface modification by grafting [22], or blending with other materials, such as PVP [23], to introduce a biofriendly interface, which may benefit the enzyme activity. In addition, polysulfone has been successfully electrospun into nanofibers [24,25], and, to our knowledge, polysulfone nanofibers were rarely used to immobilize enzymes. Hence, PSF/a biocompatible polymer electrospun composite nanofibers were adopted to immobilize lipase, in order to investigate the effect of the composite nanofibers on the behavior of the lipase.

In this study, model enzyme, lipase, was immobilized onto electrospun polysulfone nanofibers by physical adsorption, considering the hydrophobic character of PSF. The immobilization behavior and kinetic parameters were discussed with various additives in the spinning solution.

2. Experimental

2.1. Materials

Polysulfone (PSF) (from Shuguang Engineering Materials factory, Shanghai, China) possessing viscosity average molecular weight of 4.7×10^4 was used after dried in a vacuum oven. Poly(*N*-vinyl-2-pyrrolidone) (PVP) and poly(ethylene glycol) (PEG200) were used as received; their weight average molecular weights were 58,000 and 190–210 g/mol, respectively. *Candida rugosa* lipase (type VII, 26.2 U/mg protein) and *p*-nitrophenyl palmitate (*p*-NPP) were of biological grade and purchased from Sigma. Coumassie brilliant blue (G250) for the Bradford protein assay was from Urchem and BSA (Bovine Serum Albumin, BP0081) from Sino-American Biotechnology. All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of electrospun polysulfone composite nanofibrous membranes

Weighted PSF powder was dried in a vacuum oven at 80 °C for about 24 h, and then dissolved in *N*,*N*-dimethylacetamide (DMAC) at 120 °C with vigorous stirring to form homogeneous solution with PVP (or PEG200). PSF concentration was always 18 wt.% in the solution. Electrospinning was carried out using a syringe with a 1.2 mm diameter spinneret at an applied electrical potential difference of 10 kV over the 10 cm gap between the spinneret and the collector. The syringe pump was set to deliver the solution at a flow rate of 1.0 mL/h using a 20 mL syringe. It usually took 3 h to obtain a thick membrane that could be detached from the tinfoil collector. The electrospun membranes were dried in the vacuum oven at 80 °C for at least 5 h to remove the residual solvent before used.

2.3. Morphology observation

Field emission scanning electron microscopy (FEI, SIRION-100, USA) was applied to evaluated the morphology and diameter of PSF composite nanofibers at 5 kV. Before analysis, the samples were sputtered with gold using Ion sputter JFC-1100.

2.4. Immobilization of lipase onto the nanofibrous membrane by adsorption

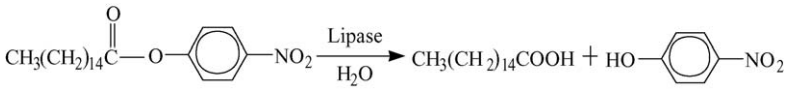
An appropriate amount of electrospun membranes was immersed in ethanol for about 3 h (to increase the wettability of the membrane), thoroughly washed with deionized water to remove the residue ethanol, and then rinsed with phosphate buffer solution (50 mM, pH 7.0). Subsequently, the pretreated membranes were submerged into the lipase solution (10 mg/mL in the buffer, 50 mM, pH 7.0) in a 25 mL beaker and shaken gently in a water bath at 30 °C for the required time. Finally, the membranes were taken out and washed with the buffer until no protein was detected in the washings.

2.5. Determination of immobilization capacity

Protein concentration in the solutions was determined with Coumassie Brilliant Blue reagent by the method of Bradford [26] using BSA as protein standard, on UV–vis spectrophotometer (756PC, Shanghai Spectrum Instruments Co. Ltd.). The amount of immobilized enzyme protein was estimated by subtracting the amount of protein determined in the filtrate and washings from the total amount of protein used in immobilization procedure. Lipase adsorption capacity of the membrane was defined as the amount of protein (mg) per gram of the fibrous membrane.

2.6. Activity assays of free and immobilized enzyme

The reaction rate of the free and immobilized lipase preparations was determined according to the method reported by Chiou [27], also described in our previous work [6]. In the standard conditions, the reaction mixture was composed of 1.0 mL ethanol containing 14.4 mM *p*-NPP and 1.0 mL phosphate buffer solution (PBS) (50 mM, pH 7.5) in an Erlenmeyer flask. The reaction was started by the addition of 0.1 mL free lipase preparation (or 25 mg immobilized lipase preparation), and the scheme is indicated as follows.



The mixture was incubated at 37 °C under reciprocal agitation at a certain stroke rate. After 5 min, the reaction was terminated