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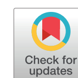
Electrospun epoxy-based nanofibrous membrane containing biocompatible feather polypeptide for highly stable and active covalent immobilization of lipase



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ABSTRACT

In this study, a novel poly (glycidyl methacrylate-*co*-methylacrylate)/feather polypeptide (P(GMA-*co*-MA)/FP) nanofibrous membrane containing reactive epoxy groups and biocompatible feather polypeptide (FP) was fabricated by electrospinning which was the first time used for the covalent immobilization of lipase. The results of FTIR spectra and SEM images of nanofibrous membrane before and after immobilization demonstrated that lipase has been successfully covalently immobilized on the nanofibrous membrane. FP was beneficial for the stabilization of the enzyme conformation which would promote the improvement of enzyme activity and stability. The P(GMA-*co*-MA)/FP-Lipase possesses a wide pH tolerance and high thermal stability, good reuse and organic solvent stability. The residual relative activity of immobilized lipase was about 38% which was treated under 70 °C for 3 h. The residual relative activity of immobilized lipase was 62% after 7 reuses and nearly 75% after being treated in methanol for 12 h. This study revealed that the biocompatible FP could be used as an additive to improve the enzyme activity and stability of immobilized enzyme on nanofibrous membranes.

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1. Introduction

Enzymes are biocatalysts and widely used in many fields for their high catalytic efficiency. However, the stability and recycling of enzymes often restrict their applications [1–3]. Immobilizing enzyme onto solid supports is an effective way to overcome these problems [4–7]. Nowadays, different kinds of nanostructured materials such as nanospheres, nanoparticles and nanofibers have been constructed and widely used in sensor, photonic crystals and enzyme immobilization [8–12]. Among of these nanostructured materials, nanoparticles and nanofibers are abundantly studied as the supports for enzyme immobilization due to their large surface area to volume ratio in recent years [13–15]. However, the difficulty of dispersion and recycling of nanoparticles restricts their application in enzyme immobilization. Nanofibrous membrane not only could solve the recycling problem but also could effectively improve the enzyme loading and catalytic efficiency. Therefore, a variety of polymers have been electrospun into nanofibrous membrane for enzyme immobilization [16,17].

Immobilization of enzymes on nanofibrous membrane can be carried out by different methods. Among of them covalent-bonded immobilization of enzymes to supports is one of the best methods to enhance the enzyme stability [18,19]. Covalent immobilization of enzymes to supports occurs between the side chain amino acids of enzyme protein and the functional groups on the supports [20–23]. The reactive groups on the supports include nitrile, amino, carboxyl and epoxy groups which have been studied extensively [24–27]. The reactive epoxy groups could offer multipoint covalent attachment between the enzyme and supports which could reduce the enzyme mobility and improve the stability. And the epoxy-base supports could react with the enzyme under mild conditions which could reduce the chemical modification of enzyme. However, enzyme activity would be reduced by the epoxy supports due to their hydrophobic and rigid surface [28,29]. Tailoring the supports surface towards biocompatibility is an effective way to improve the enzyme activity. Natural polymer materials such as chitosan and gelatin possessing good biocompatibility have been used for tailoring the surface chemistry in order to improve the enzyme activity [30,31].

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Feather polypeptide (FP) possesses excellent biocompatibility which is the hydrolysis product of keratin [32]. Our group previous research [33] revealed that a PMA-*co*-PAA/FP biopolymer nanofiber containing biocompatible feather polypeptide was fabricated by electrospinning which was used for immobilization of horseradish peroxidase exhibiting high stability and excellent reusability. In this study, a novel poly (glycidyl methacrylate-*co*-methylacrylate)/feather polypeptide (P(GMA-*co*-MA)/FP) nanofibrous membrane containing reactive epoxy groups and biocompatible feather polypeptide was fabricated by electrospinning which was used for the covalent immobilization of lipase for the first time. The influence on the enzyme loading and activity of the immobilized lipase by the different content of FP and temperature were investigated. The chemical structure and morphology of the immobilized lipase were characterized by FTIR and SEM. Lineweaver-Burke method was used to measure the kinetic parameters of the free and immobilized lipase. The influences of the catalysis reaction of immobilized lipase (pH and temperature) as well as the enzyme stability (thermal, reuse and organic solvent) were investigated.

2. Experimental

2.1. Materials

Glycidyl methacrylate (GMA), methyl acrylate (MA), sodium dodecyl sulfate (SDS), mercapto acetic acid (TGA), concentrated hydrochloric acid(HCl), ethanol, ether, acetone, sodium hydroxide (NaOH), urea, oleic acid and olive oil were of analytical reagent grade and purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Dimethylformaide (DMF) was of analytical reagent grade and purchased from Wuxi City Yasheng Chemical Co., Ltd (Jiangsu, China). Azo diisobutyronitrile (AIBN) was of analytical reagent grade and purchased from Shanghai No.4 Reagent & H.V. Chemical Co., Ltd (Shanghai, China). Candida antarctica lipase B (CALB) (1U/mg) was of biological grade and purchased from Hangzhou Novocata Biotechnology Co., Ltd (Zhejiang, China). Feather powder was supplied by Anhui Donglong Feather Manufacture Co., Ltd (Anhui, China).

2.2. Synthesis of P(GMA-co-MA) copolymer and preparation of feather polypeptide and nanofibrous membranes

GMA 15.4 g, MA 5.1 g and DMF 48 mL were fed into the three-necked round bottom flask, AIBN 0.312 g was added into the mixture under N_2 atmosphere. Then the round bottom flask was put into 70 °C water bath oscillator. After a period reaction, parahydroxyphenol was added into the mixture to terminate the reaction. The solvent was evaporated to remove, then the reaction product was dried under vacuum till the mass constant. The crude product was washed alternately by distilled water and the mixture solvent of ether and acetone (volume ratio 3:1) for three times, then the product was dried under vacuum till the mass constant to obtain the purified P(GMA-*co*-MA) copolymer.

The feather polypeptide (FP) was prepared from the feather powder. Feather powder 5 g was washed by ethanol solution with 5% HCl and dried before using which was added into the reduction processing system containing urea 14 g/L, SDS 2 mol/L and TGA 2 g/L. This mixture was kept reaction for 2 h at 80 °C under nitrogen atmosphere. The the reduced feather power was obtained after filtration, washing by water and drying under low temperature. The reduced feather power was hydrolyzed by NaOH and the hydrolysis process was as follows: the concentration of NaOH was 6 g/L and the liquor ratio was 1:50, the temperature was 100 °C and the hydrolysis time was 40 min. Then the hydrolysis solution was filtered to obtain the filtrate which was tuned the pH value to 4.2 by the HCl solution (pH = 2.0) to separate out the hydrolysis product. Then FP was obtained by filtration, washing and freeze drying.

The P(GMA-*co*-MA) copolymer with different content of FP (1.5%, 3.0%, 4.5%, 6.0% and 7.5% w/w) was dissolved in DMF to prepare spinning solution which was thoroughly stirred for 24 h by magnetic stirring until it turning uniform and transparent. The prepared spinning solution was placed into a 5 mL syringe with a inner diameter of 0.7 mm needle. The applied voltage, the flow rate of the polymer solution and the distance between needle tip and collector were controlled. The electrospinning was under the temperature of 20 °C and the humidity of 25%. After a period of time, the P(GMA-*co*-MA)/FP NFM was peeled from the aluminum foil, then freeze-dried in the vacuum drying oven.

2.3. Immobilization of lipase

A certain amount of prepared P(GMA-*co*-MA)/FP NFM was washed alternately by methanol and distilled water for three times before adding into 5 mL centrifuge tube, and CALB solution with certain pH and concentration was added. Then the centrifuge tube was put into 30 °C water bath oscillator for immobilization. The centrifuge tube was token out after a period of reaction, and then the immobilized lipase was obtained by filtering the enzyme solution. The immobilized lipase was washed by phosphate buffer solution (0.05 mol/L, pH = 7.0) until the protein couldn't be detected in the washing solution. The filtered enzyme solution and washing solution were collected for the determine of enzyme loading. The immobilized enzyme was kept at 4 °C. The detailed procedure for making electrospun P(GMA-*co*-MA)/FP NFM immobilized with lipase is schematically depicted in Fig. 1.

2.4. Determination of enzyme loading and activity

Bradford method was used to determine the enzyme loading of immobilized lipase on the NFM [34]. The enzyme protein solution 0.3 mL and Bradford working solution 3.0 mL were added into the centrifuge tube which were mixed thoroughly and then put it into the 25 °C water bath oscillator for chromogenic reaction 10 min. Phosphate buffer solution (0.05 mol/L, pH = 7.0) 0.3 mL and Bradford working solution 3.0 mL were mixed as the blank sample at the same time. The absorbance of the enzyme solution before and after immobilization was determined by the ultraviolet spectrophotometer. The enzyme concentration could be obtained from the standard curve of the absorbance and enzyme concentration. The enzyme loading was calculated by Eq. (1), the mean value was obtained by three times.

$$A_e(\%) = \frac{(C_0 - C) \times V - C_W \times V_W}{W} \times 100 \tag{1}$$

Where A_e is the enzyme loading (mg/g), C_0 is the concentration of enzyme before immobilization (mg/mL); C is the concentration of enzyme after immobilization (mg/mL); V is the volume of enzyme; C_W is the enzyme concentration of phosphate buffer washing solution after immobilization (mg/mL); V_W is the volume of phosphate buffer washing solution after immobilization (mL); W is the weight of immobilized NFM.

The catalysis activity of free and immobilized lipase was measured by using the method of hydrolyzing olive oil [35]. Olive oil 1 mL was added into 3 mL of phosphate buffer solution (0.05 mol/L, pH = 7) which was put it into the 37 °C water bath for 5 min, and then a certain amount of free or immobilized lipase was added into the olive oil solution for reaction 10 min. The reaction was stopped by addition of toluene 8 mL. The amount of generated fatty acid was measured the absorbance at 710 nm with cupric acetate as a color indicator. Oleic acid was as the fatty acid to determine the rela-

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