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Article

A magnetic biocatalyst based on mussel-inspired polydopamine and its acylation of dihydromyricetin

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ABSTRACT

A support made of mussel-inspired polydopamine-coated magnetic iron oxide nanoparticles (PD-MNPs) was prepared and characterized. The widely used *Aspergillus niger* lipase (ANL) was immobilized on the PD-MNPs (ANL@PD-MNPs) with a protein loading of 138 mg/g and an activity recovery of 83.6% under optimized conditions. For the immobilization, the pH and immobilization time were investigated. The pH and thermal and storage stability of the ANL@PD-MNPs significantly surpassed those of free ANL. The ANL@PD-MNPs had better solvent tolerance than free ANL. The secondary structure of free ANL and ANL@PD-MNPs was analyzed by infrared spectroscopy. A kinetic study demonstrated that the ANL@PD-MNPs had enhanced enzyme-substrate affinity and high catalytic efficiency. The ANL@PD-MNPs was applied as a biocatalyst for the regioselective acylation of dihydromyricetin (DMY) in DMSO and gave a conversion of 79.3%, which was higher than that of previous reports. The ANL@PD-MNPs retained over 55% of its initial activity after 10 cycles of reuse. The ANL@PD-MNPs were readily separated from the reaction system by a magnet. The PD-MNPs is an excellent support for ANL and the resulting ANL@PD-MNPs displayed good potential for the efficient synthesis of dihydromyricetin-3-acetate by enzymatic regioselective acylation.

1. Introduction

The biological catalyst lipase (glycerol ester hydrolases E.C. 3.1.1.3) is widely used in the production of biofuels, organic synthetic compounds, detergents, perfumes, cosmetics, leather, enantiopure pharmaceuticals, medical diagnostics, food and feeds [1,2]. *Aspergillus niger* lipase is a well known biocatalyst because of its wide application in the chemoselective, enantioselective and regioselective hydrolysis and synthesis of a broad range of non-natural esters [3]. However, the disadvantages of the free lipase such as poor mechanical stability, non-recyclability and difficulty in separating it from the prod-

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ucts hinder its application in industry. To overcome these problems, techniques for the immobilization of the enzyme have been widely employed [4]. For the further development of the catalytic system, the ideal immobilization technique should meet the following requirements: (1) the enzyme carrier should have a high specific surface area, good biocompatibility, be easily recyclable and can bind large amounts of the active enzyme; (2) the immobilization process should be simple, rapid and facile; (3) the immobilized enzyme should exhibit good stability in the reaction system [5].

Magnetic iron oxide nanoparticles (MNPs) have attracted interest for their properties of biocompatibility, magnetism, high surface-to-volume ratio and low toxicity [6]. They have been used as an enzyme support for their high specific surface area and easy separation from the reaction mixture by an external magnet [7]. In most cases, the MNPs require a modification or functionalization to introduce the catalyst onto the surface.

Polydopamine, which is a polymer inspired by the composition of the adhesive protein in mussels, is one of the most commonly used biomimetic materials [8]. The primary advantage of polydopamine is that it can be easily deposited on virtually all types of inorganic and organic materials by the self-polymerization of its monomer dopamine [9–11]. Furthermore, many macromolecules (such as an enzyme) containing thiol or amine can be grafted on polydopamine by the Michael addition or Schiff base reaction between catechols (a moiety of polydopamine or dopamine) and amines or thiols [12]. Therefore, it is of interest to use polydopamine to surface modify MNPs because it can be expected that the polydopamine-coated MNPs (PD-MNPs) would be an excellent support for enzyme immobilization [13] due to these advantages: polydopamine exhibits good biocompatibility, the enzyme immobilization process is simple and natural [14,15], and the immobilized enzyme can be easily and rapidly recycled with the use of a magnet.

Dihydromyricetin (DMY), a natural aglycone flavonoid, has been found to possess bioactivities with potential beneficial effects to the human body, such as anti-inflammatory, analgesic, antitussive, expectorant, antibacterial, anti-thrombotic and anti-tumor activities [16]. However, DMY is poorly soluble in aqueous and nonaqueous systems, which limits its processing and application. Our research group [17] has reported the lipase catalytic acylation of DMY, and shown that the solubility of the product in organic solvents and lipid systems was significantly improved. However, to meet the requirements of industrial production, the reusability of the lipase needed to be improved.

In this study, MNPs were prepared and surface-modified by a polydopamine coating. The polydopamine-coated MNPs (PD-MNPs) were structurally characterized in detail. The lipase from *Aspergillus niger* (ANL) was immobilized on the PD-MNPs with a high activity recovery rate and protein loading. The enzymatic properties of the immobilized lipase (ANL@PD-MNPs) were investigated systematically. The ANL@PD-MNPs were used as a magnetic recyclable biocatalyst for the regioselective acylation of dihydromyricetin (DMY).

2. Experimental

2.1. Materials

Dopamine hydrochloride was purchased from Aladdin. Ferric chloride hexahydrate ($FeCl_3·6H_2O$) and ferrous chloride tetrahydrate ($FeCl_2·4H_2O$) were obtained from Guangzhou Chemical Reagent Co. Ltd. *Aspergillus niger* lipase was purchased from Shenzhen Leveking Bio-Engineering Co. Ltd. (Shenzhen, China). DMY was obtained from Aladdin (Shanghai, China). Vinyl acetate (VA), used as acyl donor, was purchased from Sigma-Aldrich and TCI Co. Ltd. (Shanghai, China). All other reagents were analytical grade reagents and obtained from commercial sources.

2.2. Preparation of MNPs

The procedure for the preparation of MNPs was based on the conventional co-precipitation method with some modifications. In a typical experiment, 0.9 g $FeCl_2·4H_2O$ and 2.43 g $FeCl_3·6H_2O$ were dissolved in 300 mL deionized water under N_2 at room temperature. The pH of the solution was kept at 9.5 with 25% ammonia solution and vigorous stirring. After 1 h, the magnetite precipitate was collected by an external magnet and washed three times with deionized water. The precipitate was dispersed in Tris-HCl buffer (10 mmol/L, pH = 8.5) to MNPs of 2.7 mg/mL solution.

2.3. Preparation of PD-MNPs

The MNPs suspension prepared as described above was ultrasonicated for 20 min before dopamine hydrochloride (37.5 mg, 2.5 mg/mL) was added to the MNP suspension. The pH of the solution was adjusted to 8.5 by the addition of 100 mmol/L NaOH. After vigorous stirring for 1 h, the PD-MNPs were separated by an external magnet and washed three times with deionized water and then dispersed in deionized water to PD-MNPs solution of 4.2 mg/mL.

2.4. Immobilization of Aspergillus niger lipase on the PD-MNPs

Before immobilization, the PD-MNPs solution was ultrasonicated for 10 min. In order to immobilize *Aspergillus niger* lipase (ANL), the suspension of PD-MNPs was added to a buffered lipase solution. An aqueous solution of ANL (1.5 mg/mL) was prepared by dissolving the ANL powder in sodium phosphate solution (50 mmol/L, pH = 8.0). The freshly prepared PD-MNPs solution (2 mL, 4.2 mg/mL) was added to the ANL solution (1 mL, 1.5 mg/mL) at 0 °C in an ice bath. After stirring at 100 rpm for 12 h (at 0 °C), the ANL-loaded precipitate was washed with deionized water and collected. The concentration of the residual ANL in the solution and the concentration of ANL in the washings were determined by the Bradford method [18]. The amount (m) of ANL in the prepared ANL@PD-MNPs was calculated using the following equation:

$$m = m_0 - C_1V_1 - C_2V_2$$

where m_0 (mg) is the mass of ANL initially added to the solu-