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Enhancing the catalytic properties of porcine pancreatic lipase by immobilization on SBA-15 modified by functionalized ionic liquid

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ABSTRACT

The mesoporous silica SBA-15 was modified by carboxyl-functionalized ionic liquid (COOH-IL-SBA). The prepared support was used to immobilize porcine pancreatic lipase (PPL) by physical adsorption (PPL-COOH-IL-SBA) and covalent attachment (PPL-CON-IL-SBA). Enzymatic properties of the immobilized PPL were investigated in the triacetin hydrolysis reaction. It was found that carboxyl functionalized ionic liquid modification of the support surface was an effective method to improve the properties of immobilized PPL. Incorporating into the functionalized SBA-15 made PPL more resistant to temperature and pH changes, compared with PPL immobilized on parent SBA-15 (PPL-SBA). Especially, after the covalent attachment to a functionalized support, the stability of PPL was improved obviously, which retained 81.25% and 52.50% of the original activity after incubation for 20 days and four times recycling, respectively, whereas PPL-SBA exhibited only 58.80% and 27.78% of the original activity under the same conditions. In addition, physical and chemical properties of the supports and immobilized PPL were characterized by small-angle X-ray powder diffraction (SAXRD), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscope (SEM), nitrogen adsorption, nuclear magnetic resonance (NMR) and thermogravimetry (TG). The images and data confirmed chemical modification in SBA-15 and PPL immobilization on the tested support.

1. Introduction

Lipase, which exhibits excellent substrate specificity and could be operated under mild reaction conditions compared with classical chemical catalysts, is widely used in the biotechnology, pharmaceutical, food, detergent industries [1,2]. Recently, lipase immobilization has attracted significant attention in both academic and industrial areas because of the improvement in its biocatalyst performance [3–6]. The properties of immobilized lipase depend greatly on the characteristics and structure of the support materials. Mesoporous silica materials, in particular SBA-15, have received much attention as promising supports for enzyme immobilization because of their well-ordered structures, nanosized channels, and large surface areas [3,7]. Currently, various functional groups such as carboxyl, amino, and alkyl groups are being incorporated into SBA-15 to make it more suitable for enzyme immobilization and catalysis, such as conferring biocompatibility, and hydrophilicity. [8–10].

As environmentally benign "green" solvents, ionic liquids (ILs) have been used extensively in biocatalytic fields. With their properties modified via altering the cation and anion, ILs are likely to be designed for specific reaction systems. Importantly, the catalytic properties of enzymes, such as conversion rate, selectivity, stability and recoverability can be enhanced greatly by the use of ILs [11,12]. In our previous work, we synthesized and grafted alkyl functionalized IL into the surface of SBA-15 (IL-SBA), PPL was successfully incorporated into the IL-SBA by physical adsorption (PPL-IL-SBA), and the enzymatic properties have been improved [13,14]. However, the adsorbed PPL expressed poor immobilization efficiency about only 53% [14]. In addition, the immobilized PPL was not stable enough as the weak interaction forces of PPL with carriers.

To alleviate the existed problems, herein, we modified SBA-15 with specially designed carboxyl functionalized IL, and immobilized PPL by the physical adsorption and covalent methods (Scheme 1). The variation of enzymatic properties such as optimum temperature, pH, and kinetic parameters of different immobilized PPL were evaluated in the hydrolysis reaction. In addition, reusability, storage stability, and thermal stability of immobilized PPL were also investigated. We hope that the new modification method and the covalent immobilization will be useful methods for enzyme immobilization.

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2. Materials and methods

2.1. Materials

SBA-15 (pore size 6–11 nm, BET surface area 600–800 m^2/g) was purchased from Novel Chemical Technology Co., Ltd. Porcine pancreas lipase (PPL, Sigma, St. Louis, MO) was stored at 0–4 °C, the protein content was 17%. N-hydroxysuccinimide (NHS, 98%), N-ethyl-N-(3-(dimethylamino)propyl) carbodiimide hydrochloride (EDC·HCl, 98.5%), 2-(N-morpholino) ethanesulfonic acid (MES, 99%), imidazole (99%), sodium fluoroborate ($NaBF_4$) and 3-chloropropyltrimethoxysilane (98%) were purchased from Shanghai Jingchun Industry Co., Ltd. Acetone, ethanol, chloroform, ether, triacetin and other reagents used in this work were analytical grade and purchased from SCRC, China. All the solutions were prepared with distilled water.

2.2. Support modification and activation

2.2.1. Carboxyl functionalized ionic liquid modified SBA-15

We made a minor modification on the synthesis of COOH-IL-SBA according to already published procedures [15,16]. Solid SBA-15 (2 g) and 3-chloropropyltrimethoxysilane (1.99 g) were refluxed at 110 °C under a nitrogen atmosphere in 60 ml toluene. After 8 h, the white solid was filtered, washed with ethanol and ether, then dried to remove all traces of organic solvents. The solid was added to a solution of imidazole (1.36 g) in 100 ml of chloroform, and stirred at 70 °C for 24 h. The resulting mixture was filtered, and the solid was washed with ether, and dried. Then, the solid was refluxed in methylene dichloride for 24 h and dried. The obtained white solid and chloroacetic acid (1.89 g) were transferred to 100 ml toluene and refluxed at 110 °C for 24 h. The mixture was then filtered, and the solid was washed with ether. Thereafter, the solid was again refluxed in methylene dichloride for 24 h and dried. Finally, $NaBF_4$ (2.16 g) and the solid were mixed with 100 ml acetone and stirred at ambient temperature. After 72 h, the solid was collected, filtered and refluxed in methylene dichloride for 24 h. The resulting white solid was denoted as COOH-IL-SBA.

2.2.2. Preparation of CON-IL-SBA

CON-IL-SBA was prepared by using a similar procedure, as reported in Refs. [17,18]. The COOH-IL-SBA was dispersed in MES buffer (50 mM, pH 6.2), and then the mixture was added to an equal volume of NHS in MES buffer. EDC was added to the mixture to initiate the coupling of NHS to the carboxylic groups on the COOH-IL-SBA. The resulting mixture was stirred at 200 rpm for 2 h. Activated COOH-IL-SBA was rinsed thoroughly with MES buffer to remove excess EDC and NHS by filtration. The resulting white solid was named CON-IL-SBA.

2.3. Immobilization of PPL

Support solid (0.1 g; SBA-15, COOH-IL-SBA, CON-IL-SBA) was added to an aqueous solution containing 0.025 g PPL, 25 ml phosphate buffer, pH 7.5, and 25 ml distilled water. The mixture was stirred at 150 rpm and 30 °C for 2 h. Thereafter, the solid was separated from the suspension by centrifugation, and washed twice with phosphate buffer. The amount of PPL immobilized on the support was calculated by subtracting the PPL content in the supernatant from the total. Protein content was measured by the Bradford method [19]. The resulting bio-hybrids were lyophilized and named PPL-SBA, PPL-COOH-IL-SBA and PPL-CON-IL-SBA, respectively.

2.4. Activity assay

The activity of immobilized PPL was determined through the hydrolysis of triacetin, following the standard method BP 63 [7]. About 2.45 ml of triacetin and 47.55 ml of distilled water were mixed and stirred for 30 min at room temperature to prepare a triacetin emulsion. The pH of the solution was adjusted to 6.3 by adding aliquots of sodium hydroxide. Then, 0.25 g immobilized PPL hybrid was added under a moderate stirring speed. The mixture was continuously titrated with 0.05 M sodium hydroxide to maintain the pH constant at 6.3. The hydrolysis temperature was controlled at 35 °C by means of a water bath. The volume of the sodium hydroxide solution consumed in 10 min was recorded and the activity of immobilized PPL was calculated. One unit of PPL activity was defined as the amount of enzyme required to release 1 μmol of acetic acid per minute [20].

2.5. Enzymatic properties

2.5.1. Effect of temperature on activity

The temperature dependence of immobilized PPL was investigated, using the standard activity assay procedure as mentioned above, simply by adjusting the solution pH to 6.3 and varying the reaction temperature from 30 °C to 60 °C. The final consumption of sodium hydroxide solution in 10 min reflected the lipase activity.

2.5.2. Effect of pH on activity

The effect of pH on the activity of immobilized PPL was assayed by adjusting pH values ranging from 5.5 to 8.5, as the temperature was kept constant at 35 °C.

2.5.3. Reusability

The reusability test was done with four recycle runs. After each run, the immobilized PPL was recovered from the reaction system by filtration, washed with phosphate buffer solution and dried in a freeze dryer.

2.5.4. Storage stability

Immobilized PPL was stored at 4 °C. The storage stability of PPL was determined by measuring the activities of the samples every 5 days.

2.5.5. Thermal stability

The thermal stability of the immobilized PPL was assayed by immersing them in phosphate buffer at 60 °C for 0 h, 2 h, 4 h, 6 h and 8 h, respectively. After incubation, the buffer was removed carefully and the residual activity was determined under the same reaction conditions mentioned above.

2.5.6. Determination of kinetic parameters

The kinetic parameters (K_m and V_{max}) were determined by measuring the reaction rates of immobilized PPL, using different concentrations of triacetin from 10 to 70 mg/ml. The values of K_m and V_{max} were calculated from a double reciprocal plot. In all cases, the activity of immobilized PPL was determined at 5 min to avoid the possible inhibition that might take place because of the appearance of reaction products.

2.6. Characterization

Small-angle X-ray powder diffraction (SAXRD) patterns were taken on a Bruker D8 advance diffractometer, using Cu Kα radiation at 40 kV and 40 mV. The data were collected from 0.7° to 5° with a step size of 0.02°. The low-temperature N_2 adsorption experiments were carried out using an ASAP 2020 Micromeritics system. Before the measurement, samples were degassed in a vacuum at