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Covalent immobilization of triacylglycerol lipase onto functionalized novel mesoporous silica supports

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Abstract

A novel mesoporous silica material was synthesized via a silicate salt route in the presence of polyvinyl alcohol as the structure-directing agent under acidic conditions. The material was functionalized and employed as the supports (LPS-1 and LPS-2) for immobilizing triacylglycerol lipase from porcine pancreas (PPL). Not only they had a good thermal stability and reusability but also the activity recovery of LPS-1 and LPS-2 reached to 69% and 76%, respectively. The optimal pH and temperature region of the LPS supports immobilized PPL for hydrolysis of olive oil were at 8.0 and 55–60 °C. Kinetic parameters such as maximum velocity (V_{max}) and the Michaelis constant (K_m) were determined for the free and the immobilized lipase and LPS-2 immobilized PPL had the highest catalytic efficiency in the three. Meanwhile, the LPS supports exhibited many advantages than small porous materials for immobilizing PPL.

Keywords: Mesoporous silica; Covalent immobilization; Functionalized support; Activity recovery

1. Introduction

In the past few decades, many immobilization methods and support materials have been investigated (Takimoto et al., 2004; Bornscheuer, 2003). Inorganic support materials including silica gels, alumina, zeolite, and layered double hydroxides are focused due to their thermal and mechanical stability, no-toxicity, and high resistance for microbial attack (Sakaguchi et al., 2005; Tischer and Wedekind, 1999). Among them, porous inorganic materials with high specific surface area and large porous volume are a promising support for enzyme immobilization. Lots of inorganic support materials, however, have too expensive cost because of being synthesized from organism-silicon compounds, such as recently developed materials (Zhao et al., 1998; Ying et al., 1999) MCM-41, SBA-15, meso-cellular foams (MCF) and have small pore size, generally

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less than 15 nm. The small pore materials immobilization enzymes would lead to decrease the amount of enzyme loading and cumber transport of substrate and product. In addition, enzyme adsorption and entrapment on sol–gel materials are suitable techniques for enzyme immobilization, but they suffer from desorption (leakage) of the protein and diffusion limitation (Subramanian et al., 1999). Whereas functionalized silica exhibit excellent properties for enzymes immobilization (Basso et al., 2003; Lei et al., 2002). Consequently, further functionalized silica materials to introduce reactive groups and to form relative larger pores for immobilization enzymes are preferred for using silica material as supports.

In this study, we report a novel method of synthesizing the mesoporous (relative larger pore) silica material and two new ways of functionalizing the material as the supports (LPS-1 and LPS-2) for immobilized porcine pancreas lipase (PPL) (E.C. 3.1.1.3). The optimal conditions for immobilized PPL and some of the physicochemical properties of the immobilized enzyme were also investigated. In addition, the study of the properties of the LPS supports and the small porous material (MSC) were compared when they employed in immobilization PPL.

2. Materials and methods

2.1. Materials

Methane sulfonic acid, γ-chloropropyltriethoxysilane (CPTES) and 3-aminopropyltriethoxysilane (APTES) were obtained from Wuhan University Material Co. (China). Triacylglycerol lipase from Porcine pancreas (PPL) (Type Π, 177.3 U/mg protein) was purchased from Sigma Chemical Co. and silica dioxide material (MSC, average pore diameter 30 Å) was donated from Zhejiang Materials Co. (China). Other chemicals and solvents were of analytical grade and were all obtained from Tianjing Chemical Reagent Company (China).

2.2. Supports preparation

The mesoporous silica (relative larger pore, LPS) was synthesized in three steps:

First, LPS synthesized: 15 ml 0.5% (w/v) polyvinyl alcohol solution and 6 ml ethanol were poured into a stainless steel reactor and blend fully with a mechanical stirrer. After that, silicate salt (SiO_2:Na_2O 2.9, by mol, 20 Bė) and H_2SO_4 (6.0 M) were poured into the reactor synchronous with two pumps till the gel formed. Reactions proceeded at room temperature and the mixture was kept at pH 9.0 by stirring tenderly.

Second, fastness silica framework formed: The gel was aged for 24 h to prepare relatively fast silica framework. Aging was performed by using an auto controlling reactor under the conditions of pH 9.0 and a process calefactive from 35 °C to 80 °C in 4 h.

Third, the structure directing agent lustrated: After finishing the above steps, the products were washed with hot distilled water till the pH of the washing solution was at 7.0, then dried and calcined at 120 °C for 10 h and 560 °C for 6 h, respectively, so that the polyvinyl alcohol and ethanol were dispelled. The LPS synthesized following above procedures had an average pore size of 30 nm by the Barrett–Joyner–Halenda (BJH) method (Fig. 1(A)), while the specific surface area was 315 m^2/g by the Brunauer–Emmet–Teller (BET) technique. At the same time, the size of the average pore aperture can be controlled according to the amount and the molecular weight of polyvinyl alcohol. Also, in this method we have not used the relative expensive organism-silicon such as tetraethyl orthosilicate (TEOS) as the main material, and the synthesis process can be controlled easily.

After that, the LPS material was firstly pre-treated using 50% (v/v) methane sulfonic acid with ultrasonic vibration for 30 min, and was refluxed for 8 h under vigorous stirring with a magnetic stirrer, then washed with plenty of deionizing water till pH 7.0, separated by centrifugal effect, and dried at 120 °C for 6 h, successively. Secondly the pre-treated LPS (1.0 g) was further functionalized with γ-chloropropyltriethoxysilane (CPTES) and 3-aminopropyltriethoxysilane (APTES) by refluxing in anhydrous toluene for 24 h under vigorous stirring with a magnetic stirrer, respectively, which added 0.5 ml of the relevant silanizing agent and 4.0 ml toluene. Thirdly, 1.0 g CPTES functionalized LPS was reacted with 0.36 g *p*-hydroxybenzaldehyde in a mixing solution consisting of 3.5 ml toluene and 2.0 ml potash solution (0.3 M) under gentle stirring with a magnetic stirrer at 80 °C for 10 h to form support LPS-1, 1.0 g APTES functionalized LPS was reacted with 4.0 ml acrolein glycol acetal along with 2.0 ml ethanol and 0.5 ml pyridine being in the reactor under tenderly