Materials Science and Engineering C 33 (2013) 1438–1445

Contents lists available at SciVerse ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Enhanced stability of catalase covalently immobilized on functionalized titania submicrospheres

Hong Wu, Yanpeng Liang, Jiafu Shi, Xiaoli Wang, Dong Yang, Zhongyi Jiang *

Key Laboratory for Green Chemical Technology, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China

ARTICLE INFO

Article history:
Received 7 March 2012
Received in revised form 6 November 2012
Accepted 13 December 2012
Available online 22 December 2012

Keywords:
Titania
Covalent binding
Immobilized enzyme
Biocatalysis
Stability
Kinetic parameters

ABSTRACT

In this study, a novel approach combing the chelation and covalent binding was explored for facile and efficient enzyme immobilization. The unique capability of titania to chelate with catecholic derivatives at ambient conditions was utilized for titania surface functionalization. The functionalized titania was then used for enzyme immobilization. Titania submicrospheres (500–600 nm) were synthesized by a modified sol–gel method and functionalized with carboxylic acid groups through a facile chelation method by using 3-(3,4-dihydroxyphenyl) propionic acid as the chelating agent. Then, catalase (CAT) was covalently immobilized on these functionalized titania submicrospheres through 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride/N-hydroxysuccinimide (EDC/NHS) coupling reaction. The immobilized CAT retained 65% of its free form activity with a loading capacity of 100–150 mg/g titania. The pH stability, thermostability, recycling stability and storage stability of the immobilized CAT were evaluated. A remarkable enhancement in enzyme stability was achieved. The immobilized CAT retained 90% and 76% of its initial activity after 10 and 16 successive cycles of decomposition of hydrogen peroxide, respectively. Both the K_m and the V_{max} values of the immobilized CAT (27.4 mM, 13.36 mM/min) were close to those of the free CAT (25.7 mM, 13.46 mM/min).

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Enzymes are versatile biocatalysts that exhibit a number of unique advantages over conventional chemical catalysts, such as high activity, selectivity and specificity [1]. Till now, enzymes have been found in numerous applications including polymer synthesis [2], biomaterials [3], bioconversion [4], bioseparation [5], biosensors [6], etc. Development of immobilized enzymes has attracted considerable attention for many years to facilitate the reaction process and reduce the operation cost. High catalytic activity and loading capacity, easy separation and reusability are the essential requirements for immobilized enzymes [7]. Currently, adsorption, covalent binding, entrapment, encapsulation and cross-linking, or their combinations, are still the prevalent methods utilized for enzyme immobilization [8].

Compared with other immobilization methods, the covalent binding which involves the formation of covalent bonds between the reactive groups on the support and the functional groups on the enzymes offers the strongest linkage between the enzyme and the support [9]. Immobilized enzyme leaching out of reaction media and the consequent contamination to the product are thus minimized [10]. In addition, the recycling stability of immobilized enzyme is significantly enhanced. For example, catalase was covalently immobilized on nanofibers and retained 70% of its initial activity after 10 batch cycles [11]. The diffusion limitation will be reduced or eliminated when enzyme molecules are covalently immobilized on the carrier's outer surface. However, the control for covalent immobilization process is usually caustic in the past: it should meet the temperature and pH requirements of the reaction, and should be mild enough to avoid enzyme denaturation as well. Recently, there are a multitude of soft methods for covalent immobilization including the widely used EDC/NHS chemistry method [12].

Inorganic carriers usually possess high mechanical properties, thermal stability and resistance against microbial attack and organic solvents. Many kinds of inorganic solids have been used for the covalent immobilization of enzymes such as Au, SiO_2, Fe_3O_4 and TiO_2 [13–16]. Most of these inorganic carriers couldn't react with enzymes directly without surface functionalization. Therefore, surface functionalization of the carrier is necessary prior to covalent attachment of enzymes. Lü et al. synthesized epoxy group functionalized cubic mesoporous silica for covalent attachment of Penicillin G acylase. The initial specific activity of such immobilized enzyme was higher than that of enzyme immobilized by physical adsorption. The immobilized enzyme retained 72% of its initial activity after 10 batches [14]. Liu X. synthesized the magnetic particles functionalized by expoxy group for lipase immobilization. The loading capacity was 68.3 mg lipase/g support, and the activity retention of lipase was 60.4% [15]. Immobilization of catalase on modified nanofiber membranes led to an enhanced thermostability [17]. α-Amylase was covalently immobilized onto acid chloride group functionalized glass beads. The loading capacity was found to be

* Corresponding author. Tel./fax: +86 22 23500086.
E-mail address: zhyjiang@tju.edu.cn (Z. Jiang).

0928-4931/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.msec.2012.12.048

25.2 mg/g glass support. The free enzyme lost all its activity within 15 days. Immobilized enzyme lost only 20% of activity in 25 days [18]. Although the above covalent immobilization commonly led to enhanced stability, the functionalization process prior to immobilization was tedious and time-consuming, usually involving multiple steps including particle surface activation with organosilane, chemical reactions, polymerization and grafting reactions. Moreover, high temperature, addition of organic solvents and nitrogen protection were often required during the functionalization process.

It has been found that catechol and catecholic derivatives could chelate with transition metal oxide such as titania and iron oxide to form a stable salt chelate complex at room temperature rapidly [19–23]. The titania support surface could be easily modified under mild conditions at an ambient temperature through the simple chelation process. In addition, TiO_2-based materials had excellent pH and corrosion resistance, superior mechanical strength, outstanding antimicrobial performance, and in particular good biocompatibility [24]. Herein, a novel and facile approach was presented to synthesize carboxyl functionalized titania submicrospheres for enzyme immobilization. Titania submicrospheres were synthesized by a modified sol–gel method and then functionalized with carboxylic acid groups via the chelation approach using 3-(3,4-dihydroxyphenyl) propionic acid as carboxylation reagent. Catalase (CAT), as a model enzyme, was covalently anchored to the functionalized titania submicrospheres via EDC/NHS coupling reaction. The immobilized catalase was used to decompose hydrogen peroxide to water and oxygen. The activity and stability of the immobilized enzyme were investigated and compared with those of free enzyme counterpart.

2. Materials and methods

2.1. Materials

Catalase (hydrogen peroxide oxidoreductase; 2.85×10^4 units/mg protein; EC.1.11.1.6) from bovine liver, 2-(N-morpholino) ethanesulfonic acid sodium salt (MES) and Tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma. 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Shanghai Medpep Co., Ltd. Acetone, ethylene glycol, tetrabutyl titanate (TBT, >98%), hydrogen peroxide (H_2O_2, 30%), acetic acid, sodium acetate, sodium dihydrogen phosphate dehydrate ($NaH_2PO_4 \cdot 2H_2O$) and sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) were purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). 3-(3,4-Dihydroxyphenyl) propionic acid (98%) was purchased from Alfa Aesar.

2.2. Synthesis of the carboxyl acid functionalized titania submicrospheres

Titania (TiO_2) submicrospheres were synthesized by a modified sol–gel method as described in the literature [25]. 0.02 mol tetrabutoxytitanium (TBT, 6.8 ml) was added to 100 ml ethylene glycol in a round flask purged with nitrogen gas. The solution was magnetically stirred rapidly for 20 h at 30 °C. The resultant transparent solution was poured into acetone solution containing ~0.3 wt.% of water under vigorous stirring for 30 min. After aging for about 1 h, the white precipitate was collected by centrifugation at 3058 *g* for 5 min, washed with distilled water and ethanol alternatively to remove the residue ethylene glycol. The obtained TiO_2 submicrospheres with a size of 500–600 nm were dried at 80 °C for 24 h.

3-(3,4-Dihydroxyphenyl) propionic acid was used as the carboxylation reagent for the surface modification of the TiO_2 submicrospheres. A facile chemical adsorption procedure was conducted [19–23]. The TiO_2 submicrospheres were suspended in an aqueous HCl solution (pH 2.0) under ultrasonic treatment for 0.5 h. Then, the TiO_2 submicrospheres were filtered and added into an excessive 3-(3,4-dihydroxyphenyl) propionic acid aqueous solution (4 mg/ml, pH 2.0). The suspension was vigorously stirred at room temperature for 30 min to allow a complete chemisorption of catecholic salt onto the TiO_2 particle surface. The low pH condition prevented the catechol end groups of 3-(3,4-dihydroxyphenyl) propionic acid from oxidation. The modified TiO_2 submicrospheres were collected and rinsed thoroughly with deionized water. The as-synthesized carboxylic-functionalized titania (TiO_2–COOH) submicrospheres were dried at 80 °C for 24 h.

2.3. Immobilization of enzyme on functionalized titania submicrospheres

Covalent immobilization of CAT onto the TiO_2–COOH submicrospheres was performed via EDC/NHS coupling reaction [26]. The immobilization procedure consisted of two steps: EDC and NHS activation followed by coupling reaction of enzyme. First, 50.0 mg of TiO_2–COOH submicrospheres were dispersed in 10.0 ml of MES buffer solution (50 mM, pH 6.5) under ultrasonic treatment for 15 min. Then, NHS (1.5 mmol) and EDC (0.3 mmol) were added to the above suspension. The mixture was vigorously stirred at room temperature for 1 h. The NHS-activated TiO_2–COOH submicrospheres were collected by centrifugation and washed with MES buffer solution (50 mM, pH 6.5) thoroughly to remove unreacted NHS and EDC. Second, the NHS-activated TiO_2–COOH submicrospheres were dispersed in 10.0 ml of the CAT solution (5 mg/ml) prepared with MES buffer solution (50 mM, pH 6.5). The enzyme coupling reaction was allowed to proceed for 4 h at room temperature under magnetic stirring. The catalase-titania conjugates (TiO_2–CAT) were separated by centrifugation and washed thoroughly with deionized water. The immobilized enzyme was lyophilized and stored at −20 °C.

2.4. Enzyme loading capacity and activity assay

The amount of CAT immobilized was determined by measuring the initial and final concentrations of CAT within the enzyme and washing solutions using Coomassie Brilliant Blue reagent, following the Bradford's method [27]. The loading capacity was determined according to Eq. (1):

$$M\left(\frac{\text{mg enzyme}}{\text{g support}}\right) = \frac{(m - C_1 V_1)}{W} \quad (1)$$

where M represented the loading capacity; m was the amount of CAT introduced into the immobilization medium; C_1 and V_1 were the enzyme concentration and volume of the washing solution, respectively; W was the weight of the TiO_2–COOH submicrospheres. The enzyme concentration (C_1) was determined via UV–vis standard curve (correlation coefficient $R^2 = 0.998$).

The activity of free and immobilized CAT was determined by measuring the decrease in the absorbance of hydrogen peroxide at 240 nm in 5 min due to the decomposition of H_2O_2 [28].

$$2\,H_2O_2 \xrightarrow{Catalase} 2\,H_2O + O_2. \quad (2)$$

The immobilized CAT (1 mg) (or free enzyme (0.1 ml, 1 mg/ml)) was added into the H_2O_2 solution (17.64 mM) prepared in 50 ml phosphate buffer solution (50 mM, pH 7) under agitation. The decrease in absorbance at 240 nm was recorded after 5 min. One unit will decompose 1.0 μmol of H_2O_2 per min at pH 7.0 at 25 °C, measured by the decrease rate of A_{240} [29]. The relative activity of immobilized enzyme was defined as the ratio of its activity to the free form activity under the equal amount of enzyme (Eq. (3)).

$$relative\,activity(\%) = \frac{immobilized\ enzyme\ activity}{free\ enzyme\ activity} \times 100. \quad (3)$$

Download English Version:

https://daneshyari.com/en/article/10614906

Download Persian Version:

https://daneshyari.com/article/10614906

Daneshyari.com