Bioresource Technology 102 (2011) 499–506

Contents lists available at ScienceDirect

Bioresource Technology

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

Immobilization of *Candida rugosa* lipase on glass beads for enantioselective hydrolysis of racemic Naproxen methyl ester

Elif Yilmaz, Keziban Can, Mehmet Sezgin, Mustafa Yilmaz *

Department of Chemistry, Faculty of Science, Selcuk University, Konya 42075, Turkey

ARTICLE INFO

Article history:
Received 2 June 2010
Received in revised form 22 August 2010
Accepted 23 August 2010
Available online 26 August 2010

Keywords:
Candida rugosa lipase
Glass beads
Immobilization
Enantioselective hydrolysis
S-Naproxen

ABSTRACT

Candida rugosa lipase (CRL) was immobilized on glutaraldehyde-activated aminopropyl glass beads by using covalent binding method or sol–gel encapsulation procedure and improved considerably by fluoride-catalyzed hydrolysis of mixtures of $RSi(OCH_3)_3$ and $Si(OCH_3)_4$. The catalytic properties of the immobilized lipases were evaluated into model reactions, i.e. the hydrolysis of *p*-nitrophenylpalmitate (*p*-NPP). It has been observed that the percent activity yield of the encapsulated lipase was 166.9, which is 5.5 times higher than that of the covalently immobilized lipase. The enantioselective hydrolysis of racemic Naproxen methyl ester by immobilized lipase was studied in aqueous buffer solution/isooctane reaction system and it was noticed that particularly, the glass beads based encapsulated lipases had higher conversion and enantioselectivity compared to covalently immobilized lipase. In short, the study confirms an excellent enantioselectivity ($E > 400$) for the encapsulated lipase with an *ee* value of 98% for *S*-Naproxen.
© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Candida rugosa lipase (CRL) is an important industrial lipase and due to its wide substrate specificity it is successfully utilized in a variety of hydrolysis and esterification reactions. The synthesis of several pharmaceuticals could be made possible due to its high stereoselectivity and regioselectivity (Takac and Bakkal, 2007). The versatility of lipase-catalyzed reactions has made it a unique industrial biocatalyst in drug synthesis, food and flavor making, and recently in the cosmetics and perfumery industries (Hung et al., 2003). A lot of effort is still being devoted to the search for new support materials and novel techniques. The type of support as well as the method of immobilization influences the activity and operational stability of immobilized lipases. By an appropriate choice of the immobilization process, operational costs of industrial processes involving lipases can be significantly reduced. The extent of stabilization depends upon the enzyme structure, the immobilization method, and the type of support (Mateo et al., 2007; Ozmen et al., 2009a). Lipases have been immobilized on various supports by physical adsorption, covalent binding, ionic interactions or by entrapment (Yilmaz et al., 2009; Mateo et al., 2002, 2003; Guisan, 2006). A well-established sol–gel processing technique consists in hydrolyzing adequate precursors in aqueous solutions to produce soluble hydroxylated monomers, followed by polymerization and phase separation to produce a hydrated metal or semi-metal oxide hydrogel (Brinker and Scherer, 1990). Removal of water from the wet gel, which is usually accompanied by changes in the structure of the pores and of the gel's network, results in a porous xerogel. The most widely used precursors are alkyl-alkoxysilanes. These precursors were used already in the mid-1980s to prepare organically modified silicates (Ormosils) for the successful encapsulation of antibodies and enzymes (Avnir et al., 1994). Reetz et al. (2003) shown that hydrophobic sol gels may improve the activity of immobilized lipases (due to the hyperactivation of these enzymes in the presence of these surfaces). They used the 18-crown-6, Celite or Tween 80 as additive on lipase immobilization process and reported that the sol–gel lipase immobilizates were excellent catalysts in the kinetic resolution of chiral alcohols and amines, recycling without any substantial loss in enantioselectivity.

A large number of researchers have made studies with glass beads as enzyme carriers, which are inexpensive and renewable materials (Bhushan et al., 2008; Gomez et al., 2006).

α-Amylase was covalently immobilized onto phthaloyl chloride-containing amino group functionalized glass beads. The immobilized α-amylase exhibited better thermostability than the free one (Kahraman et al., 2007). The glutaraldehyde technique is very versatile and may be used in very different fashions (Betancor et al., 2006; Migneault, 2004). However, in terms of stabilization, the treatment with glutaraldehyde of proteins previously adsorbed in supports bearing primary amino groups offers in many cases very good results, because permit the crosslink between glutaraldehyde molecules bound to the enzyme and glutaraldehyde molecules bound to the support. However, it implies the chemical modification of the whole enzyme surface (López-Gallego et al.,

* Corresponding author. Tel.: +90 332 2233873; fax: +90 332 2412499.
E-mail address: myilmaz42@yahoo.com (M. Yilmaz).

0960-8524/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2010.08.083

2005). Gomez et al. (2006) showed that soybean and horseradish peroxidases were immobilized on glutaraldehyde-activated aminopropyl glass beads to characterize these immobilized derivatives and to compare the behavior of the free and immobilized enzymes.

In our previous work (Yilmaz et al., 2010), sporopollenin was utilized to encapsulate lipases, and the prepared enzyme by polycondensation with tetraethoxysilane (TEOS). The catalytic properties of the immobilized lipases were evaluated into model reactions, i.e. the hydrolysis of *p*-nitrophenylpalmitate (*p*-NPP), and the enantioselective hydrolysis of racemic Naproxen methyl ester that was studied in aqueous buffer solution/isooctane reaction system. The results indicated that the sporopollenin based encapsulated lipase particularly had higher conversion and enantioselectivity compared to the sol–gel free lipase. In this study, excellent enantioselectivity ($E > 400$) has been noticed for most lipase preparations ($E = 166$ for the free enzyme) with an *ee* value 98% for *S*-Naproxen. Recently (Sahin et al., 2009) *C. rugosa* lipase was encapsulated by polycondensation of tetraethoxysilane (TEOS) and octyltriethoxysilane (OTES) in the presence of calix[*n*]arene, calix[*n*]–NH_2 and calix[*n*]–COOH ($n = 4$, 6, and 8) compounds as additive. For the encapsulated CRL-catalyzed hydrolysis of racemic Naproxen methyl ester in aqueous phase/isooctane biphasic medium, temperature, pH of the aqueous phase and calix[*n*]arene-based additives were also found to have important effects on the conversion and enantioselectivity.

In the present work glass beads (GB) were selected as immobilization matrix because of their excellent mechanical properties and, because they can be modified to include a variety of functional groups. In this work, the alkylamine-support was obtained by aqueous silanization, which, in contrast to silanization in organic solvents, appears to produce a monolayer of silane across the carrier surface of high hydrolytic stability, providing greater support and a sufficient degree of enzyme loading (Janowski et al., 1991). The modified support was then activated with glutaraldehyde and covalently linked to the enzymes via available amino functions (Weetal, 1969). Glutaraldehyde has been proposed as a versatile and powerful way to immobilized enzymes (López-Gallego et al., 2005; Betancor et al., 2006), permitting very different situations (using preactivated supports, adsorption plus covalent immobilization, direct covalent immobilization, use of dimmer or monomers, or using enzymes adsorbed on aminated support may be possible to adsorb and later cross-linking the enzyme to the support). Cross-linking was avoided by washing the support thoroughly before adding the enzyme to remove all excess glutaraldehyde (Gomez et al., 2006). Moreover, the *C. rugosa* lipase was encapsulated within a chemically inert sol–gel support prepared by polycondensation with tetraethoxysilane (TEOS) and octyltriethoxysilane (OTES) in the presence and absence of glutaraldehyde-activated aminopropyl glass beads as additive and explored the effect of these materials in the enantioselective hydrolysis of racemic Naproxen methyl ester. The effect of temperature, pH and thermal/storage stability was also investigated.

2. Methods

2.1. Materials

A commercial lipase powder (lyophilizate) such as CRL type VII was obtained from Sigma-chemical Co., (St. Louis, MO) used in the immobilization. Non-porous glass beads with average diameters of 9–13 μm were supplied by Aldrich. Bradford reagent, bovine serum albumin 99% (BSA), *p*-nitrophenylpalmitate (*p*-NPP) was purchased from Sigma-chemical Co., (St. Louis, MO). Acetone and ethanol were provided by Merck (Darmstadt, Germany). All aqueous solutions were prepared with deionized water that had been passed through a Millipore Milli-Q Plus water purification system. All other chemicals (Merck, Darmstadt, Germany) were of analytical grade and used without further purification.

2.2. Surface activation of glass beads

After some modification, silanization of the glass beads (GB) has been made according to the published procedure (Ozmen et al., 2009b). About 10 g of glass beads were pre-cleaned by treatment in aqueous solution of 2 M NaOH (50 mL). The glass beads were gradually heated to reflux temperature for 15 min with continuous stirring. The suspension was poured into deionized water (200 mL) at room temperature, and the glass beads were filtered, washed with deionized water to neutral and dried in a vacuum oven at 120 °C for 24 h.

2.3. Silanization of glass beads

The activated glass beads (5 g) were transferred to a 250 mL flask containing dry toluene (20 mL) and then nitrogen was bubbled through the mixture. After that (3-aminopropyl)-triethoxysilane (APTES, 5 mL) was added to the slurry and the mixture was stirred at 75 °C for 8 h under nitrogen atmosphere. The glass beads were filtered, washed successively with toluene (50 mL), dichloromethane (40 mL), and acetone (40 mL), and air-dried for 24 h, giving APTES-treated glass beads (Ozmen et al., 2009b).

2.4. Treatment with glutaraldehyde of modified glass beads

The modified glass beads (5 g) were suspended in aqueous 15% glutaraldehyde (GA) (50 mL) in 50 mM phosphate buffer, pH 7, stirred at 25 °C for 6 h, and filtered. The residue was washed with deionized water (50 mL), and dried under vacuum at 70 °C for 24 h to give the glutaraldehyde-treated glass beads (GB-GA).

2.5. Immobilization of lipase (covalent immobilization)

About 1.5 g of the support with GA was suspended by dissolving 0.45 g of lipase powder in 10 mL, 50 mM phosphate buffer solution (pH 7). Flask was incubated at 30 °C at 180 rpm. After 6 h, the support was washed thoroughly with 50 mM phosphate buffer (pH 7). Immobilized enzyme was analyzed for expression of bound lipase activity. The preparation was then lyophilized and stored at 4 °C until use.

2.6. Sol–gel encapsulation of CRL

Sol–gel encapsulated lipases were prepared according to a modified method of Reetz et al. (2003). *C. rugosa* lipase (CRL) (60 mg) was placed in a 50-mL Falcon tube (Corning) together with phosphate buffer (390 μL; 50 mM; pH 7) and the mixture was vigorously shaken with a Vortex-Mixer. The GB-GA was included. Then 100 μL of aqueous polyvinyl alcohol (PVA) (4% w/v), aqueous sodium fluoride (50 μL of 1 M solution) and isopropyl alcohol (100 μL) were added, and the mixture homogenized using a Vortex-Mixer. Then the alkylsilane (2.5 mmol) and TMOS (0.5 mmol; 74 μL; 76 mg) were added and the mixture agitated once more for 10–15 s. Gelation was usually observed within seconds or minutes while gently shaking the reaction vessel. Following drying overnight in the opened Falcon tube, isopropyl alcohol (10 ± 15 mL) was added in order to facilitate removal of the white solid material (filtration). The gel was successively washed with distilled water (10 mL) and isopropyl alcohol (10 mL). The resulting encapsulated lipases were lyophilized and stored at 4 °C prior to use.

Download English Version:

https://daneshyari.com/en/article/682256

Download Persian Version:

https://daneshyari.com/article/682256

Daneshyari.com