Journal of Molecular Catalysis B: Enzymatic 116 (2015) 134–139

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Immobilization of bovine carbonic anhydrase on glycidoxypropyl-functionalized nanostructured mesoporous silicas for carbonation reaction

Xiaoyao Fei, Shaoyun Chen, Chunjie Huang, Dai Liu, Yongchun Zhang*

State Key Laboratory of Fine Chemistry, School of Chemical Engineering, Dalian University of Technology, Dalian 116024, PR China

ARTICLE INFO

Article history:
Received 9 January 2015
Received in revised form 16 March 2015
Accepted 22 March 2015
Available online 30 March 2015

Keywords:
Epoxy-functionalized SBA-15
Carbonic anhydrase
Immobilization
CO_2 sequestration

ABSTRACT

Bovine carbonic anhydrase (BCA) has been immobilized on glycidoxypropyl-functionalized SBA-15 (GFS) for biomimetic carbonation reaction. GFS was characterized by N_2 adsorption–desorption, Fourier transform infrared analysis, ^{13}C and ^{29}Si CP MAS NMR spectroscopy. The immobilization time and material dose were optimized. The influences of pH value and temperature on the activity of free and immobilized BCA, storage stability and reusability of immobilized BCA were investigated using a para-nitrophenyl acetate (p-NPA) assay. The kinetic parameters K_m and K_{cat}/K_m for free and immobilized BCA were found to be 2.4 mM and 896.4 M^{-1} s^{-1}, and 3.1 mM and 757.4 M^{-1} s^{-1}, respectively. It was observed that the immobilized BCA can retain around 91% of its initial activity up to 30 days at 4 °C, which showed higher storage stability than free BCA. Reusability studies suggested that immobilized BCA could keep high activity after 20 cycles of carbonation reaction. BCA immobilized on GFS (BCA-GFS) were used for hydration of CO_2. The CO_2 sequestration capacity in terms of conversion CO_2 to calcium carbonate was quantified by organic elemental analysis. The amount of $CaCO_3$ precipitated over GFS-BCA was nearly the same as that precipitated over free BCA, 227 mg of $CaCO_3$/mg of BCA-GFS as compared to CO_2 sequestration capacity of 241 mg of $CaCO_3$/mg of BCA.

1. Introduction

Carbon dioxide (CO_2) is the major composition of greenhouse gas which potentially influences climate change due to the rapid accumulation in atmosphere [1]. Biological carbon sequestration might be an eco-friendly method to reduce anthropogenic CO_2 from industrial gas among all technologies based on different physical and chemical processes, such as absorption, adsorption, membranes, and cryogenics [2–5]. Enzymes as the effective catalysts have advantageous features in industrial applications due to high specific selectivity and efficiency under ambient conditions [6]. Carbonic anhydrase (CA, E.C. 4.2.1.1) is a Zn-metalloenzyme which catalyzes the reversible hydration of CO_2 into bicarbonate. It is ubiquitously found in living organisms [7]. The high turnover number of 10^6 s^{-1} of CA makes it a suitable candidate for biomimetic CO_2 sequestration [8]. Bond et al. [9] developed an integrated system in which the enzyme CA was first employed to accelerate the hydration of CO_2 for converting it into carbonate. Mirjafari et al. [10] studied the effect of bovine carbonic anhydrase on the hydration of CO_2, and then converted the carbonate to calcium carbonate. Liu et al. [11] investigated the precipitation of $CaCO_3$ from produced waters in the presence of CA enzyme. Vinoba et al. [12] tested CO_2 absorption rate and absorption heat using CA within amine absorbents, with an emphasis on the role of CA enhancing the reaction rate.

However, there are some practical limitations in the application of enzymes in their native form, such as high costs, low thermal and operational stability, narrow pH range, difficulties in recovery and reuse [13–15]. Immobilization of the enzymes onto porous silica supports provides one of the most attractive concepts to overcome these drawbacks [15]. Nanostructured mesoporous silicas (MPs) are very attractive for enzymatic immobilization process, since they possess ideal characteristics to enzyme immobilization, including the large surface area, uniform in size, narrow pore size distribution, and their thermally, mechanically and chemically stability [14–19]. However, there was a very significant (35–52%) leaching observed

Abbreviations: BCA, bovine carbonic anhydrase (BCA); GPTMS, 3-glycidyloxypropyltrimethoxysilane; GFS, glycidoxypropyl-functionalized SBA-15; BCA-GFS, BCA immobilized on GFS; p-NPA, para-nitrophenyl acetate; p-NP, para-nitrophenol; Tris, Tris(hydroxymethyl)aminomethane; TEOS, tetraethylorthosilicate; TMB, trimethyl benzene.

* Corresponding author. Tel.: +86 411 84986332; fax: +86 411 84986332.
E-mail address: zalidy5518@vip.sina.com (Y. Zhang).

http://dx.doi.org/10.1016/j.molcatb.2015.03.016
1381-1177/

under the reaction conditions of enzyme immobilization, which using pure siliceous mesoporous as supports to immobilize enzyme by adsorption method [18,20,21]. One approach to reducing the degree of enzyme leaching from pure siliceous mesoporous is using organic functionalization of the MPs surface to strengthen interaction of the enzymes with the matrix through covalent binding method, which could tightly fixed enzyme molecules, thus, enzyme leaching is minimized and enzyme contamination of the product can be avoided [15,22]. Yiu et al. [22] investigated the enzyme immobilization on different types of functionalized SBA-15, thiol, chloride, amine, and carboxylic acid functional groups, which were attached by siloxypropane to the siliceous surface of SBA-15, and the resulting immobilized enzyme catalysts were shown to be active, stable and reusable. However, to induce covalent linkage with enzymes, epoxy-activated supports are presumably the most accessible ones to perform very easy immobilization, as they are very stable in aqueous media and able to directly form covalent bond with different protein groups including amine, thiol, and phenol ones without using of cross-linking agent under mild condition [23,24]. Therefore the operation of immobilizing enzyme on glycidoxypropyl-functionalized mesoporous materials is very simple, which makes it quite suitable for enzyme immobilization. Although some researchers have described using epoxy functionalized supports to immobilize enzyme [25,26], to the best of our knowledge, glycidoxypropyl-functionalized SBA-15 (GFS) for CA immobilization has not yet been reported in the published literature.

In the present study, CA isolated from bovine was immobilized on the GFS. Detail studies, such as the influences of immobilization time and material dose on the immobilization capacity of GFS, the effects of pH value and temperature on the activity of free and immobilized BCA and the storage stability of free and immobilized BCA have been investigated. Moreover, reusability of immobilized BCA was also examined. Carbonation study has also been carried out to establish proof that the immobilized BCA could be effectively used for the sequestration of CO_2 through mineral carbonation. The organic elemental analysis technique has been used for quantification of calcium carbonate.

2. Materials and methods

2.1. Materials

Bovine carbonic anhydrase (BCA) that separated from bovine erythrocyte was purchased from Worthington Biochemical Corporation. Triblock copolymer (poly(ethylene oxide)-block-poly-(propylene oxide)-block-poly(ethylene oxide), EO_{20}-PO_{70}-EO_{20}, MW 5800) (P123), Tetraethylorthosilicate (TEOS), trimethyl benzene (TMB), 3-glycidyloxypropyltrimethoxysilane (GPTMS), Tris(hydroxymethyl)aminomethane (Tris), para-nitrophenyl acetate (p-NPA), para-nitrophenol (p-NP), acetonitrile, anhydrous calcium chloride, $Na_2HPO_4{\cdot}12H_2O$, $NaH_2PO_4{\cdot}2H_2O$, and the Bradford reagent were all purchased from Aladdin. All compounds were of analytical grade and were used without further purification.

2.2. Methods

2.2.1. Synthesis of GFS material

The synthesis procedure of SBA-15 was referenced to the literature [27]. Briefly, 4 g of P123 was dissolved in 150 mL of 1.6 M HCl at 40 °C and stirred until complete dissolution. The reaction mixture was stirred for 2 h after addition of 0.3 g of TMB. The silica precursor TEOS (9.2 mL) was added to the mixture, which was stirred at 40 °C for 10 min subsequently. The mixture was further allowed for 24 h at 40 °C statically, and then aged at 120 °C for 24 h. The resulting mixture was then cooled, filtered and washed with deionized water until neutral prior to drying under a vacuum at 100 °C for 24 h. The recovered as-synthesized sample was calcined in air at 550 °C for 6 h with a heating rate of 1 °C/min. Glycidoxypropyl-functionalized SBA-15 was obtained by refluxing 1 g of SBA-15 and 50 mM of GPTMS in absolute ethanol under N_2 atmosphere for 24 h. The product was filtered, washed with ethanol, and then dried overnight at 80 °C under a vacuum. The obtained materials were designated as GFS.

2.2.2. Immobilization procedure and enzyme assay

About 5 mg GFS was blended with 10.0 mL of BCA in buffer (0.1 mg/mL BCA in 100 mM sodium phosphate, pH = 7.0). The mixture was kept in the shaker at 25 °C for 2 h, and then centrifuged and washed with 100 mM sodium phosphate buffer (pH = 7.0) at 25 °C for 30 min. The filtrate was used for the further assay. The concentration of BCA (before and after immobilization) was calculated by the Bradford method [28]. The product was denoted as BCA-GFS (Fig. S1). Esterase activity of the BCA was measured according to the method described by Sahoo et al. [29] with a slight modification. The assay system consisted of 0.3 mL of enzyme in a 1 cm spectrophotometric cell containing 2.4 mL of Tris buffer (50 mM, pH = 8.5) and 0.3 mL of p-NPA dissolved in acetonitrile. The changes in absorbance at 348 nm were measured with a UV-vis spectrophotometer for 5 min at 30 s intervals, before and after adding enzyme. Different concentrations of p-NPA (1.0, 2.0, 3.0, 4.0, and 5.0 mM) were tested at fixed enzyme concentration ([BCA] = 0.05 mg/mL) to determine the kinetic parameters. BCA-GFS was assayed at as same concentrations of substrate as free BCA. A Michaelis–Menten and Lineweaver–Burk plot has been used to calculate K_m and K_{cat}/K_m of the enzyme. One unit of enzyme activity was expressed as 1 μmol p-NP released per minute at room temperature. Blank experiments were also conducted to estimate the self-dissociation of p-NPA in each assay solution.

2.2.3. Determination of optimum immobilization time and material dose

The influences of immobilization time and material dose on the amount of BCA immobilization were investigated by incubating the reaction mixture in the immobilization time range between 1 h and 5 h and in the material dose range between 2 mg/10 mL and 20 mg/10 mL.

2.3. Characterization

The nitrogen adsorption–desorption isotherms were measured on a Quantachrome AUTOSORB-1-MP system at −196 °C. Prior to each adsorption measurement, the samples were evacuated at 80 °C under vacuum ($p < 10^{-5}$ mbar) in the degas port. The specific surface area (S_{BET}) was determined from the linear portion of the BET curve, the pore volume was calculated using the BET plot corresponding to the amount of nitrogen gas adsorbed at the last adsorption point ($P/P_0 = 0.95$), and the pore size distribution was estimated using the Barrett–Joyner–Halenda (BJH) method. Fourier transform infrared (FT-IR) spectra of the samples were recorded at room temperature on a Bruker-vector-22 in a range of 4000–500 cm^{-1}. ^{13}C CP MAS NMR spectra and ^{29}Si CP MAS NMR spectra were recorded on Agilent-NMR-vnmrs500 125.7 and 99.3 MHz with a sample spinning frequency of 5 kHz. Organic elemental analysis was carried out on Elmentar Vario EL III.