Journal of Molecular Catalysis A: Chemical 296 (2008) 28–35

Contents lists available at ScienceDirect

Journal of Molecular Catalysis A: Chemical

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

Microperoxidase-11-NH_2-FSM16 biocatalyst: A heterogeneous enzyme model for peroxidative reactions

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ARTICLE INFO

Article history:
Received 30 May 2008
Received in revised form 15 August 2008
Accepted 30 August 2008
Available online 5 September 2008

Keywords:
Mesoporous silica
Microperoxidase-11
Nanobiocatalyst
Modification
Heterogeneous model enzyme

ABSTRACT

FSM16 mesoporous silicate and its chemically modified samples were synthesized. Then the relevant nanobiocatalysts consisting of Fe(III)protoporphyrin(IX) (Hemin, Fe(III)PPIX), microperoxidase-11 and horseradish peroxidase were obtained via direct immobilization of the biocatalysts in the nanopores of amine modified FSMs. The prepared catalysts were characterized by XRD, ASAP and diffuse reflectance UV/Vis techniques. The performances of the obtained peroxidase model nanostructures were evaluated by some typical test reactions, such as oxidation of ABTS, *ortho*-methoxyphenol (guaiacol) and peroxidatic synthesis of indophenol and *N*-antipyryl-*p*-benzoquinoneimine. Kinetic parameters including initial reaction rates, rate constants, V_{max}, turnover number, Michaelis constant and catalytic efficiency were obtained and compared to those of Fe(III)PPIX/MCM41 (as a blank) and homogeneous native horseradish peroxidase (HRP). Results showed that MP-11-NH_2-FSM16 nanobiocatalyst is able to mimic horseradish peroxidase with a K_m value of $55.45 \pm 1.29\,\mu M$ with respect to ABTS and guaiacol (as the reducing substrates of HRP). The prepared nanobiocatalysts with high catalytic efficiencies about $10^8\ M^{-1}\ min^{-1}$ showed high peroxidatic activity for oxidation and conversion of aromatic substrates.

1. Introduction

The discovery of ordered mesoporous materials (OMMs) prepared by the liquid surfactant templates has been a significant breakthrough in the catalysis by porous materials. OMMs offer unique potential for the immobilization of catalysts and biocatalysts regarding their ordered, homogeneous and large pores. Biocatalysis plays important role in clean production of energy and fuels, fine chemicals, pharmaceuticals, and green processes. Mostly, heterogeneous biocatalysts consist of catalytically active species localized on the surface of a solid support, preferably a porous solid to achieve improved rate, yield and efficiency. In contrast to heterogeneous catalysts, homogeneous ones have disadvantages, such as lower stability and less successful separation from the reaction mixture. The advantages of heterogeneous catalysts over the homogeneous ones are facile separation and recovery, regeneration and reusability of the catalyst and possibility of its using at extreme conditions such as acidic, alkaline and nonaqueous media, higher temperatures and pressures. Green chemistry principles and environmental issues are pushing the new industrial processes towards biotechnological-based industries. Indeed, as the ultimate goal in catalysis science and engineering [1] and in these environmentally conscious and economically pressured days, homogeneous catalysts preferentially need to be replaced by the alternative green solid catalysts. Hence, the selected strategy is immobilization of the homogeneous catalyst on an insoluble support, referred to as *heterogenization of homogeneous catalyst* [2].

Enzymes as the potent biocatalysts with high selectivities, have been used in the food industry for hundreds of years. Currently, enzymes are becoming increasingly important in sustainable technology and green chemistry [3]. The application of an enzyme for a given reaction is often hampered by major limitations such as high cost and low stability. If an enzyme is immobilized on a rigid support, this limitation can be overcome since the immobilized biocatalyst enables easy separation, reusability, and simple operation [4]. Some immobilized enzymes such as glucose isomerase and penicillin G acylase (PGA) have reached large-scale industrial applications [5,6] and immobilization of other enzymes has been of great interest in research [7].

Abbreviations: HRP, horeseradish peroxidase; MP-11, microperoxidase-11; OMMs, ordered mesoporous materials; ABTS, 2,2-azino-di-3-ethyl-benzothiazoline-(6)-sulphonic acid; CTAB, cetyl trimethylammonium bromide; Fe(III)PPIX, iron(III) protoporhyrin IX; Gl, glutaraldehyde; S, aromatic reductant substrate.

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doi:10.1016/j.molcata.2008.08.018

Today, ordered mesoporous materials with uniform pore sizes (2–30 nm); high surface area (~1000 m^2/g) and large pore volume (~1 cm^3/g) are well known [8–10]. Among these materials, FSM16 [8], MCM41 [9], MCM48 [9] and SBA15 [10] have been extensively studied for catalyst immobilization. MCM48 possesses a three-dimensional, bicontinuous cubic pore structure [9]. The availability of ordered mesoporous materials has opened up unprecedented opportunities for immobilizing biocatalysts. The pore size of these ordered materials can be precisely controlled over a wide range and using these solid supports, the heterogeneous single-site catalysis can be achieved [3,11,12]. Over the past ten years, research and development in using mesoporous silicas as carriers for catalysts has advanced rapidly. This topic has been comprehensively reviewed elsewhere [3,11–15].

Depending on the type of the interaction between the catalyst and the solid support, four common methods for the immobilization of homogeneous catalysts can be introduced: covalent binding, electrostatic interaction, adsorption, and encapsulation.

Covalent binding is by far the most frequently used method for immobilization of homogeneous catalysts. Immobilization via electrostatic ionic interactions is conceptually simple, and is a facile method for immobilizing ionic catalysts or those catalysts that can be ionized under the immobilization conditions. While the adsorption method is simple, it tends to yield an unstable catalyst because of the weak interaction between catalyst and support. Encapsulation is the only catalyst immobilization method that does not require any interaction between the catalyst and the support, but the size of the pore-openings in the support must be smaller than the kinetic size of the immobilized catalyst. In addition to these immobilization methods, cross-linking and entrapment of enzymes can also be used [5–7,16–18]. The advantages and disadvantages of the different methods for enzyme immobilization have been discussed elsewhere [19–23]. In general, for efficient immobilization the support can first be functionalized (preferentially). Ordered mesoporous silicas provide excellent opportunities for the immobilization of both homogeneous and enzyme catalysts via covalent binding because of the availability of well-defined silanol groups [24,25]. The major advantage of covalent binding is the stability of the immobilized enzyme, thus minimizing enzyme leaching [26]. Covalent binding of α-L-arabinofuranosidase to an amino-functionalized, bimodal mesoporous silica support revealed that not only the biocatalyst works under a wider range of experimental conditions (lower pH and higher temperatures), but also possesses a higher resistance toward glucose and ethanol in comparison with the free enzyme [27]. Using SBA15 materials with different surface functionalities (–SH, –Ph, –Cl, $–NH_2$, and –COOH) to immobilize trypsin resulted in solving the leaching of the enzyme by using SBA15 functionalized with –SH, –Cl, and –COOH.

It must be noted that the harsh conditions employed during covalent binding can potentially alter the enzyme conformation, thus lowering the enzymatic activity. In addition, binding of the active sites of the enzyme with a support may result in a total loss of the activity. It has been found that PGA physically adsorbed onto the pores of SBA15 silica retains up to 97% of the activity of free PGA, while PGA covalently attached onto the pores of oxirane-grafted SBA15 retains only 60% of the activity [19]. Nevertheless, such a loss in activity can be compensated by the advantages of immobilized enzymes, such as easy separation from the reaction medium, potential reuse, and the possibility of using the immobilized enzyme in a packed-bed or fluidized-bed reactor.

Peroxidase models are capable of catalyzing some peroxidative reactions such as oxidation, epoxidation and hydroxylation of organic compounds [28–30]. Fe(III)protoporphyrin(IX) (Fe(III)PPIX, hemin) as a known catalytic active site finds in hemoproteins like hemoglobin, myoglobin, cytochromes and peroxidases [31,32]. There is great interest in metalloporphyrins for analytical, synthetic and biotechnological purposes. Homogeneous metalloporphyrins and hemoenzymes have low stability in water solutions so that they may become inactivated at extreme conditions (severe acidic or alkaline pHs, high temperatures, high concentrations of peroxide (>2 mM) and in the presence of reactive solvents) [33,34]. The stability of a biocatalyst during synthesis/purification processes and in operational conditions is of vital importance in biotechnology. Several strategies are in hand to increase operational stability of a biocatalyst including the use of stabilizing additives, immobilization, encapsulation, crystallization and media engineering [19,34,35]. Encapsulation of iron(III)protoporphyrin biocatalyst and preparation of a heterogeneous peroxidase model catalyst via direct synthesis of iron(III)protoporphyrin/MCM41 is reported previously [36].

In order to improve the enzyme loading and immobilization, first, FSM16 should be functionalized preferably by using the covalent binding method. The most useful surface functional groups are thiols, carboxylic acids, alkyl chlorides and amines [14]. Other functional groups, such as vinyls, have been found to modify the enzyme's environment by increasing the hydrophobicity of the support surface [37].

Present work discusses a facile procedure for preparation of heterogeneous peroxidase and peroxidase model enzymes along with comparing and characterization of their kinetic behaviour and potential applications for organic synthesis purposes.

2. Experimental

2.1. Materials

Microperoxidase 11 (MP-11, sodium salt), horseradish peroxidase, Fe(III)PPIX (hemin chloride) and cetyl trimethylammonium bromide (CTAB) were obtained from Sigma. Tetramethylammonium hydroxide (TMAOH), hydrogen peroxide, phenol, guaiacol and 2,2-azino-di-3-ethyl-benzothiazoline-(6)-sulphonic acid (ABTS) were purchased from Merck and used without further purification.

Kanemite (a hydrated layer sodium silicate, $NaHSi_2O_5{\cdot}3H_2O$) was prepared from a $NaOH/SiO_2$ mixture (molar ratio 1:1). NaOH was dissolved in a small amount of water, and then the solution was diluted with ethanol so that silica could easily be dispersed result in formation of homogeneous slurry. After evaporation at 50 °C, the resulting paste was dried at 100 °C and finally calcined at 700 °C for 6 h.

All solutions were prepared using deionized water (Barstead NanoPure D4742; E.R. = 18 MΩ).

The obtained molecular sieves were characterized by X-ray diffraction using a Philips-PW model 1840 X-ray diffractometer with Cu Kα radiation.

Furthermore, diffuse reflectance UV/Vis and atomic absorption spectroscopy techniques were used for comparative investigation of various prepared catalysts using Varian Cary 500 and Varian AA200 spectrophotometers, respectively.

2.2. Methods

2.2.1. Synthesis of FSM16 and its modified samples

FSM16 was prepared based on the direct synthesis method as previously reported [38–42]. The silica-surfactant self-assembly process occurs both at the solid–liquid and the liquid–vapor interfaces. Typically, 10.0 g (0.06 mol) kanemite ($NaHSi_2O_5{\cdot}3H_2O$) was dispersed in 100.0 mL of deionised water and then mixed with 100.0 mL 0.2 M CTAB containing 0.5 M triethylbenzene. pH of the solution was adjusted at 9–10 and the mixture was stirred at room