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Immobilization of horseradish peroxidase in phospholipid-templated titania and its applications in phenolic compounds and dye removal

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ABSTRACT

In this study, horseradish peroxidase (HRP) was encapsulated in phospholipid-templated titania particles through the biomimetic titanification process and used for the treatment of wastewater polluted with phenolic compounds and dye. The encapsulated HRP exhibited improved thermal stability, a wide range of pH stability and high tolerance against inactivating agents. It was observed an increase in K_m value for the encapsulated HRP (8.21 mM) when compared with its free counterpart. For practical applications in the removal of phenolic compounds and dye by the encapsulated HRP, the removal efficiency for phenol, 2-chlorophenol, Direct Black-38 were 92.99%, 87.97%, and 79.72%, respectively, in the first treatment cycle. Additionally, the encapsulated HRP showed better removal efficiency than free HRP and a moderately good capability of reutilization.

1. Introduction

Enzymes are able to catalyze many chemical processes under most benign experimental conditions [1]. In this way, enzymes could be excellent catalysts for a much more sustainable chemical industry [2]. However, enzymes have some limitations for nonbiological applications [3,4]. Thus, for many industrial applications, enzymes have to be immobilized, via very simple and cost-effective protocols, in order to improve the properties of enzymes, such as activity, stability, and selectivity [5,6]. Over the last several decades, three types of methodology for enzyme immobilization have been reported in scientific literatures: via binding to or encapsulation in an inorganic or organic polymer [7,8], or by cross-linking the enzyme molecules [9]. No single method has been emerged as the standard for enzyme immobilization and ongoing efforts are striving to optimize these methods to render them adequate for specific applications [10,11].

Compared to most organic polymers, the silica matrix exhibits higher mechanical strength, enhanced thermal stability, and negligible swelling in organic solvents. Thus, silica matrix made by sol–gel process, has emerged as a promising platform for encapsulation of enzymes to be used in biocatalysis, biosensors, and biomedical fields [12,13]. However, the relative harsh reaction conditions and long aging time [14] of the traditional sol–gel process may induce the deactivation of enzymes. Thus, the biomimetic silicification process, which provides a rapid, low temperature method for silica precipitation, has emerged as a versatile tool for preparing excellent supports for enzyme immobilization. Until now, a remarkable diversity of enzymes has been encapsulated in bioinspired silicas, and it is reasonable to envisage that this technological impact of bioinspired encapsulation will continue to grow [5].

Although much of the research effort on biomimetic silicification has been taken, exploration of phospholipid-templated silica matrix seems to have received less attention. This approach can overcome the disadvantages of entrapment techniques, including the leaching of adsorbed biomolecules, the chemical degradation of the anchoring bond of covalently attached enzymes, and diffusion limitations of substrates and products. This is clearly confirmed by various authors [15–18]. Furthermore, this phospholipid-templated approach can eliminate specific enzyme–silica interactions during the silica formation process, and can produce more active biocatalyst than those prepared by trapping enzymes directly in silica hydrogels [16].

In comparison with silica-based materials, titania-based materials have attracted great interest in a number of fields [19–21], owing to their unique chemical and physical properties, including stable chemical structure [22], good biocompatibility, relative high conductivity, and environmentally benign nature [23]. Additionally, titania is an amphoteric oxide, allowing it to be an anion and

Abbreviations: HRP, horseradish peroxidase.

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cation exchanger at acidic and alkaline pH, whereas silica can only act as a cation exchanger [14,24]. Thus, the titania material has been investigated and proved to be an ideal enzyme carrier [25–27].

In the past years, the so-called biomimetic titanification, which typically refers to the mimic of biological synthesis process to form titania, has emerged as a versatile tool for preparing titania materials [28,29]. This facile and green process for titania synthesis can be controlled at near-neutral pH, ambient temperature within a short period [23,24,27]. Many proteins and peptides, such as silicatein [30], lysozyme [31], protamine [27], and R5 peptide [32] have been successfully employed for the formation of titania through the biomimetic titanification process [33]. In contrast to these reports, we report the first examination of using dodecylamine to catalyze the hydrolysis and subsequent polycondensation of a water-stable alkoxide-like conjugate of titanium to yield titania under ambient conditions for enzyme immobilization. To eliminate the specific enzyme-titania interactions, generate 3-D open mesoporosity for facile diffusion of substrates in biocatalysis process, and then produce more active biocatalyst, phospholipid was used as template. To the best of our knowledge, this is the first report that concerned the preparation of phospholipid-templated titania particles by using biomimetic titanification and as an efficient matrix for enzyme immobilization.

Phenolic compounds and direct dyes are widely distributed in the wastewater of the textile industry and they can be highly harmful toward aquatic life and humans. [34,35]. Therefore, a number of techniques including adsorption, chemical oxidation, solvent extraction and biodegradation aimed at preferential removal of the phenolic compounds [36–38] and dyes [39,40] from wastewaters have been developed. Among these treatment technologies, the use of oxido-reductive enzymes such as horseradish peroxidase (HRP) to catalyze the removal of pollutes has become increasingly important [41–44].

Thus, in the present work, HRP was encapsulated in phospholipid-templated titania particles that induced by dodecylamine through the biomimetic titanification process. The effect of pH, thermal stability, tolerance against inactivating agents, and kinetic parameters of the encapsulated HRP were investigated. The removal of phenolic compounds (phenol and 2-chlorophenol) and dye (Direct Black-38) by the free and encapsulated HRP was also investigated. The results presented here not only open a novel avenue for immobilizing enzymes but also provide methods that can be readily adapted for a range of metal oxide synthesis. Additionally, the relatively low price and easy availability ensure dodecylamine as a promising titania-precipitating agent for large-scale utilization.

2. Materials and methods

2.1. Materials

Horseradish peroxidase (HRP, EC. 1.11.1.7, 150 U/mg) was purchased from Source leaves Biotechnology Co. (shanghai, China). Soybean lecithin, phenol, 2-chlorophenol and H_2O_2 30% (w/v) were purchased from Jiang Tian Chemical Technology Co. (Tianjin, China). Titanium (IV) bis (ammonium lactato) dihydroxide (Ti-BALDH) and Direct Black-38 were purchased from Sigma Chemical Co. (St. Louis, USA). Other chemicals were of analytical grade and were used as received without further purification.

2.2. Preparation of the encapsulated HRP

After optimization in the preliminary experiment, a typical experimental procedure for encapsulation of HRP in titania was described as follows: A first solution of lactose (0.05 g) in 7.2 mL phosphate buffer pH 7.0 containing HRP (4.32 mg) was prepared at 37 °C. It was added slowly under vigorous stirring to a second solution, also prepared at 37 °C, composed of lecithin (0.7 g) and dodecylamine (0.05 g) in 5.2 g ethanol.

Then 2 mL of Ti-BALDH (0.25 mol/L, pH 7.0) solution was then added slowly to the above mixture, the biomimetic titanification process was preceded for 15 min. A gentle stirring was maintained until the titania particles were formed. To remove lecithin, the resulting particles were washed with Triton X-100 (7.5%) and distilled water, then the encapsulated HRP was obtained.

Surface morphology of the encapsulated HRP was investigated through scanning electron microscopy (JSM-6700F, JEOL, Japan). Samples were dried by rinsing with anhydrous acetone, sputter-coated with gold prior to the examination. The diameters of the titania particles were determined by using a Brookhaven Instruments BI200SM dynamic light scattering (DLS) system.

The enzymatic activity of HRP was measured by Worthington method [37]. One unit of HRP activity (U) was defined as the amount of HRP required to hydrolyze 1 μmol of H_2O_2 in 1 min at 25 °C and pH 7.0.

2.3. The properties of the encapsulated HRP

The effect of pH on the activity was evaluated by incubating free and encapsulated HRP with equal activity in phosphate buffers (0.1 M) for 2 h at pH 3.0–9.0 under 25 °C. Then the HRPs were taken out and the residual activities were measured. The relative activity was calculated as the ratio between the activity at each pH and the maximum activity.

The study on thermal stability of free and encapsulated HRP was carried out by measuring the residual activity incubated over different times in the phosphate buffer (0.1 M, pH 7.0) at 50 °C and 60 °C.

The tolerance capacity of free and encapsulated HRP against inactivating chemicals was tested by incubating HRP in different denaturing solutions, namely 10 μM of $CaCl_2$, $CuCl_2$, $BaCl_2$, $MnCl_2$, 8 mM of H_2O_2, 25% (v/v) methanol, and 25% (v/v) acetone (0.1 M PBS, pH 7.0) at 25 °C for 30 min. The residual activities were measured by Worthington method.

The kinetic model used in this study was based on the Michaelis–Menten equation ($1/V$ vs $1/[S]$). The experimental initial reaction rates (Vi) were determined from the plot of the consumption of H_2O_2 as a function of time. The concentration of phenol was 0.17 mM, and the concentration of H_2O_2 was varied from 0.7 mM to 2.0 mM in the reaction medium.

2.4. Enzymatic removal of phenolic compounds and dye

Two phenolics (phenol and 2-chlorophenol) and one dye (Direct Black-38) were employed in this research. The phenol (8 mM), 2-chlorophenol (60 mM) and Direct Black-38 (120 mg/L) degradation were studied under the same H_2O_2 concentration (8 mM) with the encapsulated HRP (130 U) at 25 °C. The solution was stirred to ensure full contact of the substrates and the encapsulated HRP.

The residual phenolic compunds were measured on the basis of colorimetric method with potassium ferricyanide and 4-aminoantipyrine with a UV–vis spectrophotometer at 505 nm in 100 mL round bottom flask [45]. Dye decolorization was measured based on the maximum absorbance of Direct Black-38 at 550 nm [9].

3. Results and discussion

3.1. Characterization of the encapsulated HRP

With the objective of synthesizing titania materials as enzyme host from Ti-BALDH, we used lecithin/dodecylamine mixed-micelle as the template. Scheme 1 shows the building process of the phospholipid-templated titania particles and HRP encapsulation. In this process, lecithin and dodecylamine were anticipated to play three roles. First, the lecithin/dodecylamine mixed-micelle that worked as template for the titania formation could generate 3-D open mesoporosity for facile diffusion of substrates in biocatalysis process. Second, dodecylamine played the role of nucleophilic catalyst to promote the condensation of titania precursor in the synthesis process, without generating elevated pH, which was indeed advantageous to avoid enzyme denaturation. Third, the lecithin could protect the enzymes from unfriendly environment. Fig. 1 shows typical SEM image of the obtained HRP-containing titania particles (encapsulated HRP). It can be seen that the samples were formed by an agglomeration of microspheres, which was similar with the silica-based materials reported by various authors [15–18]. DLS results (Fig. S1) showed that the titania particles had an average size of about 190 nm. After optimization, the immobilization yield would be 70.51% and the enzyme activity recovery was 56.31%.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.enzmictec.2013.11.005.