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Highly efficient covalent immobilization of catalase on titanate nanotubes

Qinghong Ai[a], Dong Yang[a,c], Yuanbing Li[a], Jiafu Shi[b,c], Xiaoli Wang[b], Zhongyi Jiang[b,c,*]

[a] Key Laboratory of Systems Bioengineering of Ministry of Education, School of Chemical Engineering and Technology, Tianjin University, Tianjin 30072, China
[b] Key Laboratory for Green Chemical Technology of Ministry of Education, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China
[c] Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300072, China

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ABSTRACT

In this study, titanate nanotubes (TNTs) with desirable biocompatibility and hydrophilicity have been synthesized by a facile and cost-effective alkaline hydrothermal method, and used to immobilize the enzyme. The characterization results reveal that the prepared TNTs have a regular tubular morphology with a length about 100–180 nm and an outer diameter about 10 nm, and a BET specific surface area of 305.4 $m^2 g^{-1}$. Catalase (CAT), as the model enzyme, was pre-modified by 3-(3,4-dihydroxyphenyl) propionic acid (3,4-diHPP) via 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) coupling chemistry, and then covalently immobilized on the TNTs surface by the chelation of catechol groups with Ti^{4+} ions. It is found that TNTs exhibits excellent performances as the immobilized supporter of enzyme: the enzyme loading is as high as 820 mg g of support^{-1}; the relative activity of immobilized enzyme is about 60% of that of free enzyme; the immobilized CAT demonstrates enhanced storage and recycling stability.

1. Introduction

Nanobiocatalysis, which refers to the incorporation of enzymes into nanostructured materials, has attracted much attention based on the nanotechnology progress in recent years [1–5]. Nanostructured materials have been recognized excellent enzyme scaffolds, because they offer the special and fascinating characteristics for balancing the key factors that determine the biocatalyst efficiency, including high specific surface area, minimized mass transfer resistance, and effective enzyme loading over their bulk counterparts [1,4]. Till now, various nanostructures, such as nanoparticles [6], nanofibers [7], nanotubes [8], nanoporous media [9], and graphene [10] have been explored as supporters for enzyme immobilization, demonstrating superior and unique application potentials.

Among all of the nanostructured materials, much effort has been dedicated to the research and development of nanotubes, especially carbon nanotubes (CNTs), as the enzyme immobilization supporter due to their exceptional properties, for instance, large aspect ratio, extraordinary mechanical and thermal properties [11–14]. Wang et al. [15] immobilized NADH oxidase on the CNTs surface based on the specific interaction between His-tagged NADH oxidase and functionalized single-walled CNTs, which demonstrated high enzyme loading capacity and stabilities. Zhang et al. [16] investigated the interactions between adsorbed catalase and CNTs with different morphologies and surface functionalities, indicating that the enzyme activity was significantly influenced by the unique curvature of the nanomaterials. However, unmodified CNTs incline to exhibit high hydrophobicity and severe aggregation, and thus difficult to be compatible with organic or biological materials. One of the most popular approaches for functionalizing CNTs involves the introduction of carboxylic groups onto their surfaces via the strong acid treatment, nevertheless the chemical inertness of CNTs dramatically hampers their potential applications [17]. In comparison, titanate nanotubes (TNTs) which possess considerable specific surface area, demonstrate attractive biocompatibility, hydrophobicity and special chelation interactions with catechol groups [18,19]. Although TNTs have been recently applied in the bio-sensing [20–22], there are few reports concerning their utilization in enzyme immobilization. Furthermore, the functional hydroxyl groups on the TNTs surface are expected to provide an aqueous-like environment, and stabilize the structure of immobilized enzymes.

* Corresponding author at: Key Laboratory for Green Chemical Technology of Ministry of Education, School of Chemical Engineering and Technology, Tianjin University, Tianjin 30072, China. Tel.: +86 22 2740 6642; fax: +86 22 2740 6642.
E-mail address: zhyjiang@tju.edu.cn (Z. Jiang).

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In this study, TNTs were employed as the supporter for pursuing the efficient enzyme immobilization. TNTs were synthesized by a facile and cost-effective alkaline hydrothermal method, and utilized for the covalent immobilization of enzyme for the first time. TEM, XRD, XPS, Raman, BET and FTIR analysis methods were used to determine the morphology and chemical characteristics of the as-prepared TNTs. Catalase (CAT) as the model enzyme was modified by 3-(3,4-dihydroxyphenyl) propionic acid (3,4-diHPP) by EDC/NHS method, and then immobilized on the TNTs surface through the chelation of catechol groups with Ti^{4+} ions. The enzyme loading efficiency and corresponding catalytic activity were measured, and the thermal, storage, and recycling capability of immobilized CAT were also experimented.

2. Materials and methods

2.1. Materials

Catalases (EC 1.11.1.6 from bovine liver; 2.48×10^4 U mg of protein^{-1}); 2-(N-morpholino) ethanesulfonic acid sodium salt (MES) and tris (hydroxymethyl) aminomethane (Tris) were purchased from Sigma–Aldrich Chemical Co. Ltd. 3-(3,4-Dihydroxyphenyl) propionic acid (98+%) was purchased from Alfa Aesar. 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Shanghai Medpep Co. Ltd. Hydrogen peroxide (30.0%) was purchased from Guangfu Co. Ltd. (Tianjin, China). Sodium hydroxide was purchased from Jiangtian Co. Ltd. (Tianjin, China). Rutile TiO_2 powder (99.8%, 60 nm) was purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China). Other chemicals were of analytical grade. The water used in all experiments was prepared in a Millipore Milli-Q purification system.

2.2. TNTs synthesis

Regular titanate nanotubes were prepared by a hydrothermal method described by Geng et al. [23]. In a typical procedure, nano-sized rutile TiO_2 powders (2 g) were firstly dispersed in 85 mL NaOH solution (10 mol L^{-1}). Then, the suspension was transferred into a sealed Telfon-lined container, and statically heated for 72 h at 130 °C. The white precipitate was obtained after centrifuged, washed with excess deionized water, and soaked in abundant HCl (0.1 mol L^{-1}) for 10 h, followed by washing with deionized water until pH 7.0. Finally, alcohol was used to disperse the white precipitate in order to displace water on the TNTs surface and enable the formation of aggregation-free nanotubes.

2.3. Covalent immobilization of CAT on the TNTs surface

As shown in Scheme 1, CAT was modified by grafting 3,4-diHPP coupled with EDC and NHS as activators, and then immobilized on the TNTs surface based on the chelation interaction between Ti^{4+} and catechol group of 3,4-diHPP. Briefly, 3,4-diHPP (20 mg), NHS (36 mg) and EDC (60 mg) were added orderly in an MES solution (pH 6.5, 15 mL) under continuous magnetic stirring. Then, a CAT solution was added in the above mixed solution, reacting for 4 h at 4 °C. Through the dehydration–condensation reaction between —COOH and —NH_2 radicals, 3,4-diHPP was grafted on the CAT molecules. Subsequently, 50 mg TNTs were dispersed in 5 mL MES buffer solution (50 mmol L^{-1}, pH 6.5) under ultrasonic treatment for 15 min, and then mixed with the above modified CAT solution for 30 min under stirring vigorously. Finally, CAT immobilized on the TNTs surface (TNTs-CAT) was obtained after centrifugation and thoroughly washing with deionized water for removing free CAT adsorbed physically on the TNTs surface.

2.4. Characterization

The morphology of the prepared TNTs was performed on a transmission electron microscopy (TEM) (IEM-100CX II) instrument. X-ray diffraction (XRD) pattern was obtained with a Rigaku D/max 2500 v/PC X-ray diffractometer in the range of 5–40° at the speed of 5° min^{-1} (Cu Kα, 40 kV, 200 mA). The Raman spectrum of TNTs was recorded by a Bruker FS100 FT-Raman spectrometer with a liquid N_2-cooled super InGaAs detector. The spectrum was excited with a diode pumped YAG laser (532 nm) with a power of 100 MW. The specific surface area and pore volume were recorded by nitrogen absorption–desorption isotherm measurements performed on a Micromeritics Tristar 3000 gas adsorption analyzer. The pore size distribution was determined by the nitrogen isotherms based on the Barret–Joyner–Halenda (BJH) method. The element-mapping analysis was carried out by an energy dispersive X-ray spectroscope (EDS), which was directly connected to the TEM. X-ray photoelectron spectroscopy (XPS) was conducted to examine surface elemental composition of TNTs. FTIR spectra were acquired on a Nicolet-560 spectrometer. UV–vis spectra were acquired using a UV spectrophotometer (Hitachi U3010). Thermogravimetric analysis (TGA) was recorded on a Perkin–Elmer thermogravimetric analyzer.

2.5. Enzyme activity

The catalytic activity of CAT was determined spectrophotometrically at 240 nm by using H_2O_2 as the substrate. Briefly, 0.1 mL of free CAT or as-prepared TNTs-CAT (0.1 mg mL^{-1}) was added to 20 mL H_2O_2 (20 mmol L^{-1}) solution prepared by a Tris–HCl buffer (50 mmol L^{-1} pH 7.0) under stirring for 3 min. The absorbance decrease at 240 nm caused by H_2O_2 decomposition was recorded after CAT or TNTs-CAT being added in the above solution for 3 min. One unit of catalytic activity was defined as the decomposition of 1 μmol H_2O_2 per min at 25 °C and pH 7.0. The concentration of CAT was determined by Brandford method, and the catalytic activity of free CAT and TNTs-CAT were given as U mg of protein^{-1}. The relative activity (%) of TNTs-CAT was calculated by comparing with the free CAT under equal enzyme amount under the same conditions (Eq. (1)).

$$\text{Relative activity } \% = \frac{\text{activity of TNTs-CAT}}{\text{activity of free CAT}} \times 100 \quad (1)$$

2.6. Immobilization efficiency, kinetic parameter and stability of TNTs-CAT

2.6.1. Immobilization efficiency

CAT solutions with different initial concentrations were modified to covalently bind on the surface of prepared TNTs. The amount of CAT immobilization was determined by the TGA analysis, and the corresponding relative activity of TNTs-CAT with different loading capacities was also calculated.

2.7. Leakage rate

Fifty milligram TNTs-CAT with a loading efficiency of 820 mg g of support^{-1} was incubated in 20 mL Tris–HCl buffer (50 mmol L^{-1}, pH 7.0) with vigorous magnetic stirring for 72 h. Then, the supernatant was collected by centrifugation, and the concentration of CAT in supernatant was examined by the Bradford's method. The leakage rate of TNTs-CAT was calculated by the following equation:

$$\text{Leakage ratio } \% = \frac{C_i V_i}{M_T} \times 100 \quad (2)$$