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High efficient chromogenic catalysis of tetramethylbenzidine with horseradish peroxidase immobilized magnetic nanoparticles

S. Kim [1], J. Lee [1], S. Jang, H. Lee, D. Sung, J.H. Chang *

Korea Institute of Ceramic Engineering and Technology, Jinju 660-031, South Korea

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ABSTRACT

The improving method of enzyme activity can be a great way to enhance the economics of biocatalytic processes. This work reported the highly efficient bio-nanocatalyst (HRP@Si-MNPs) with horseradish peroxidase (HRP) immobilization on the silica-coated magnetic nanoparticles (Si-MNPs). These bio-nanocatalysts were prepared by the reversible immobilization of silica-coated magnetic nanoparticles and HRP protein via electrostatic interactions. The binding capacity of HRP onto Si-MNPs could reach 5 ± 1.5 μg per 1 mg of Si-MNPs. Subsequently, the chromogenic decomposition of tetramethylbenzidine (TMB) was evaluated by HRP@Si-MNPs and free HRP. As a result, HRP@Si-MNPs appeared darker-blue color than that of free HRP at same substrate concentration. In addition, the apparent kinetic constant value (V_{max}) of immobilized HRP was 260 μmol min^{-1} mg $protein^{-1}$, which was 88-folds higher than the free HRP. Therefore this approach opens new opportunities to lower manufacturing costs in large-scale industries of environment and biotechnology.

1. Introduction

Horeseradish peroxidase (HRP), found in the roots of horseradish, is a heme-containing oxidoreductase which catalyzes the reductive cleavage of hydrogen peroxide (H_2O_2) by an electron donor [1]. This enzyme is ideal in many industry because of cheap production, relative stable property and wider variety of substrates than other oxido-reductive enzymes [2]. HRP has been used extensively in environmental, industrial biotechnologies and biomedicine including removal of phenols from polluted water, organic synthesis, and analytical diagnosis [3–5].

Immobilization of enzymes to solid supports is especially useful in industrial large scale because of reusability with the aim of reducing the production cost by efficient recycling and control of the process. Nanoparticles are considered to be an excellent support for enzyme immobilization due to their maximum surface area per unit mass and high enzyme loading capability [6]. Especially, iron oxide nanoparticles as a magnetic material have been widely used in many fields because of easy and effective separation of proteins, DNA, cells, biochemical products [7].

The most common procedures of immobilization on nanoparticles can be divided as reversible (ionic bonding or physical adsorption) and irreversible (covalent bonding) [8]. During the irreversible immobilization procedure, enzymes usually lose their activities because of blocking or distorting the active sites of enzymes. However, the reversible immobilization enhances the activity of the enzyme. Recently, self-assembled reversible immobilization of horseradish peroxidase with bare magnetic nanoparticle (MNPs), referred to as bio-nanocatalysts (BNCs), showed enhanced peroxidase activity [9]. They demonstrated that overall activities of the BNCs are improved dramatically only when the enzymes are in close association with the MNPs. However, BNCs used naked Fe_3O_4 nanoparticles which tend to aggregate to minimize the surface energies. In addition, this bare iron oxide nanoparticles are easily oxidized in air, generally resulting in loss of magnetism.

Here, we developed highly efficient chromogenic bio-nanocatalysts with HRP immobilization on the silica-coated magnetic nanoparticles to overcome these limitations because these nanoparticles are hydrophilic, bio-compatible and stable in most biosystems (Fig. 1) [10]. Our bio-nanocatalysts were prepared by the electrostatic interactions between silica-coated magnetic nanoparticles and HRP. Subsequently, we showed the faster chromogenic reaction than free HRP enzyme for tetramethylbenzidine (TMB) as a function of the H_2O_2 concentration and catalytic

* Corresponding author.
E-mail address: jhchang@kicet.re.kr (J.H. Chang).
[1] These authors contributed equally to this work.

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time, respectively. Finally, we demonstrated the apparent values of the Michaelis–Menten parameters to analyze this enhanced peroxidase activity.

2. Experimental

2.1. Materials

Horseradish peroxidase (M = 44,000, 162 units/mg), 3,3′5,5′-tetramethyl benzidine (TMB), iron(II) chloride tetrahydrate (99%), iron(III) chloride hexahydrate (98%), tetraethyl orthosilicate (98%) and phosphate buffered saline (PBS) were purchased from Sigma–Aldrich chemical. They were used as received without further purification.

2.2. Instrumentation

The magnetic nanoparticles and silica coated magnetic nanoparticles were characterized by powder X-ray diffraction (XRD) using a Rigaku D/MAX-2500 diffractometer with filtered Cu-Kα radiation, and the data were collected for 2θ of 20.0°–80.0°. The hydrodynamic sizes and zeta-potentials of MNPs and Si-MNPs were measured using an DLS 8000 dynamic light scattering apparatus. The particle size and morphology of silica coated magnetic nanoparticles were determined by transmission electronic microscopy (TEM) using a JEM-4010 (JEOL) at an accelerating voltage of 400 kV. The magnetization of silica coated magnetic nanoparticles at room temperature up to 10 KO_e was measured by vibrating sample magnetometer (VSM) using a VSM 4179 (Oxford Instruments). Fourier transform infrared (FT-IR) spectroscopy was used to identify the functionalized MNPs@SiO_2. The FT-IR spectra were recorded on a V-460 (JASCO) FT-IR spectrometer using KBr pellets. Spectra were obtained at a resolution of 4 cm^{-1}, and the wavenumber range from 4000 cm^{-1} to 650 cm^{-1}. UV–vis spectra of the samples were recorded on a UV–vis spectrophotometer (V-550, JASCO, Japan).

2.3. Synthesis of the HRP@Si-MNPs

The synthesis of Si-MNPs was conducted according to our previous paper [11]. The synthesized Si-MNPs were dispersed in 20 mM citrate buffer (pH 5) and were mixed with 5 mg/mL of HRP solution at RT for 1 h. Then the mixture was collected by centrifugation (12,000 rpm, 20 min) and freeze-drying.

2.4. Electrostatic surface view of HRP

Three-dimensional structural data of HRP (PDB ID: 1GWU) was used as model for the electrostatic charge surface. The representations of the surface charge with the positive charge (blue) and the negative charge (red) were generated with PYMOL program.

2.5. Peroxidase activity assay of free and immobilized enzyme

The analysis of free HRP was achieved using hydrogen peroxide and TMB. The reaction was initiated by an addition of 5 μL hydrogen peroxide with concentration ranging between 0.5 mM and 8.8 mM to the mixture of 974 μL phosphate buffer solution (pH 7.4) containing 42 mM TMB, and 1 μL of 11.4 μM HRP. The oxidation reaction process was traced by measuring the UV–vis absorbance of characteristic TMB oxidate at 650 nm. Assay of HRP@Si-MNPs (2 mg) was performed under the similar condition.

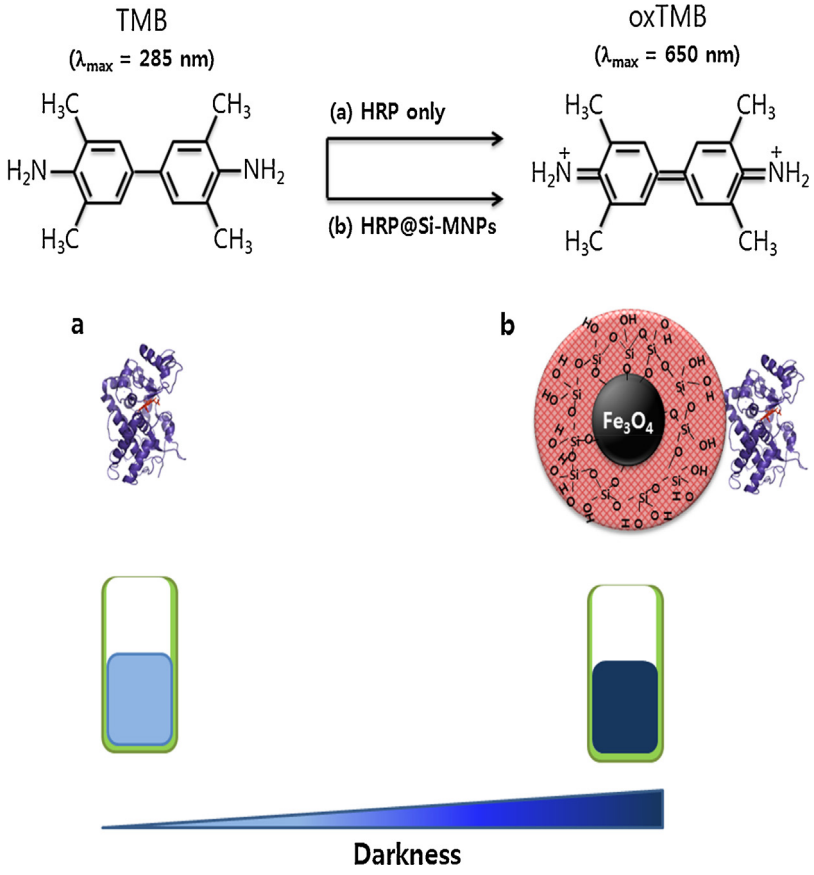

Fig. 1. (a) Horseradish peroxidase (HRP) (b) the reversible immobilization of silica-coated Fe_3O_4 nanoparticles (Si-MNPs) and HRP.