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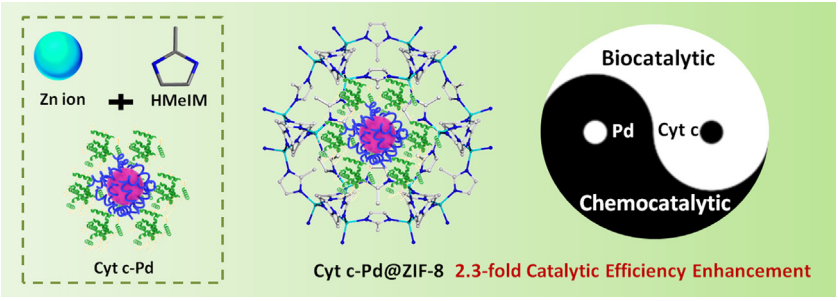
Palladium-mediated hybrid biocatalysts with enhanced enzymatic catalytic performance via allosteric effects

Huan Xia, Xue Zhong, Zhixian Li *, Yanbin Jiang

School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou 510640, China

GRAPHICAL ABSTRACT



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ABSTRACT

High activity and stability of immobilized enzymes have been constant pursuits and critical challenges for decades. Herein, Cytochrome c (peroxidase, Cyt c) and its corresponding enzyme mimic (Pd nanoparticles) were combined and successfully embedded into a zeolitic imidazolate framework-8 (ZIF-8) to enhance the enzymatic catalytic performance using a biomimetic mineralization approach. Owing to allosteric effects of Cyt c-Pd complexes, the as-synthesized Cyt c-Pd@ZIF-8 composites exhibit an increased turnover number (approximately 2.4-fold for k_{cat}) and an enhanced catalytic efficiency (approximately 2.3-fold for k_{cat}/K_M) compared to free Cyt c; also the shielding effect of ZIF-8 endows enzyme with improved resistance against harsh conditions (e.g. high temperatures and organic solvents). The strategy, which integrates enzyme with its enzyme mimic derived from transition metal nanoparticles to enhance enzymatic catalytic performances, may provide a versatile and facile technique for designing highly efficient and multi-functional bio-catalysts.

1. Introduction

Enzymes, as catalysts of nature with prominent catalytic activity and high regioselectivities, hold great promise for applications in fine chemicals, pharmaceutical production, bio-diesel and bio-sensing [1,2]. However, harsh conditions such as organic solvents and high temperatures often result in severe denaturation of free enzymes, which impedes their practical applications in industry [3,4]. Enzyme immobilization has become a viable strategy to circumvent these disadvantages, because it not only retains enzyme activity but also improves operability and stability of free enzymes [5,6]. Recently, Zeolitic Imidazolate Frameworks (ZIFs), as a type of porous materials which are reticularly comprised of linking metal node and organic ligands by strong bonds, have emerged as an ideal platform for enzyme immobilization [7,8].

To date, approaches of integrating enzymes with ZIFs to synthesize enzyme-ZIF composites mainly include surface attachment, covalent linkage, co-precipitation, and biomimetic mineralization.

* Corresponding author.
E-mail address: lizhx@scut.edu.cn (Z. Li).

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Typically, surface attachment and covalent linkage allow ZIFs to be pre-synthesized. Surface attachment often results in enzyme leakage since enzymes are physically absorbed onto ZIFs surface through weak interactions [9]. By covalent linking functional groups of enzyme surfaces with ZIFs, enzyme leakage could be largely relieved, sacrificing its catalytic activity [10]. Co-precipitation and biomimetic mineralization are *in situ* approaches to encapsulate enzyme during the process of preparing ZIFs. These approaches, which are conducted under mild conditions, can maintain biological function and shield enzymes against denaturing conditions [11–14]. Tsung et al. embed catalase into ZIFs (ZIF-8 and ZIF-90) crystals via a *de novo* approach to obtain the catalase-ZIF composites, which exhibits retained enzymatic activity even when treated under a denature reagent and high temperatures [11,12]. Falcaro et al. develop a biomimetic mineralization approach to encapsulate horseradish peroxidase into ZIF-8, which protects the enzyme from inhospitable environments such as boiling water and N, N-dimethylformamide [13].

Recent studies have proved that enzyme-ZIF composites render a more stable enzyme with largely retained activity [11,12]. However, the contradiction of activity and stability could not be ignored after immobilization due to hindered mass transfer or deactivated enzyme [15,16]. Previously, Pd, Pt and Au nanoparticles (NPs) are reported to exhibit peroxidase-like performance [17,18]. Palladium (Pd), in particular, shows biocompatible and efficient catalytic activity for wide range of oxidation reactions under mild conditions [19,20]. Therefore, to further enhance enzymatic activity and utilize the shielding effect of ZIFs, we designed a rational synergic catalytic system (Cyt c-Pd complexes) based on Cytochrome c (Cyt c, a robust and low-cost peroxidase) and peroxidase mimic (Pd NPs), then integrated it into ZIFs by biomimetic mineralization. We also demonstrated allosteric effects of Pd NPs on Cyt c in aqueous solution. Cyt c molecules turn out to be much more active when bound to Pd NPs. By encapsulating Cyt c-Pd complexes into ZIF-8, the as-synthesized Cyt c-Pd@ZIF-8 exhibits both high catalytic efficiency and stability.

2. Experimental section

Materials. Zinc nitrate hexahydrate (98%), Fluorescein isothiocyanate (FITC) (90%), Cytochrome c (CAS#9007-43-6, from bovine heart), 2-methylimidazole (HMeIM) (99%), $NaBH_4$ (98%), $PdCl_2$ (99%), 2, 2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid ammonium salt) (ABTS) (98%) and polyvinylpyrrolidone (PVP) (Mw 55000) were purchased from Sigma-Aldrich.

Synthesis of Pd nanoparticles. 5.0 mg PVP (Mw 55000) and an acidic solution (in 0.5 M HCl) of $PdCl_2$ precursor were dissolved in 10 mL deionized water to give a metal concentration of 1 mM. After the solution was sonicated at room temperature for 10 min, 0.1 mM $NaBH_4$ was added. Then, the colour of the solution changed from faint yellow to grey. The solution obtained was dialyzed in deionized water for 12 h. The final solution was stored at 4 °C prior to use.

Synthesis of Pd-Cyt c@ZIF-8 composites. 50 μL Cyt c water solution (10 mg/mL) was added into 0.5 mL as-synthesized Pd NPs solution under stirring at room temperature for one hour, then the above solution and $Zn(NO_3)_2$ solution (150 mM, 0.5 mL) were added into the solution containing 2-methylimidazole (1 M, 4 mL). After incubation at room temperature for 30 min, the product was obtained by centrifugation and washed with deionized water. The process of preparing Cyt c@ZIF-8 was similar to that of Pd-Cyt c@ZIF-8 without adding Pd NPs. Enzyme encapsulation efficiency and enzyme loading are calculated as follows.

$$\text{Enzyme encapsulation efficiency } (\%) = \frac{(m - c \cdot V)}{m} \times 100\% \quad (1)$$

$$\text{Enzyme loading } (\%) = \frac{(m - c \cdot V)}{W} \times 100\% \quad (2)$$

where m (mg) represents the weight of Cyt c initially added to the solution, c (mg/mL) and V (mL) represent the Cyt c concentration and volume of the supernatant after immobilization respectively, *W* (mg) represents the total weight of Cyt c-Pd@ZIF-8 composites.

Characterization Methods. All of the enzyme concentration quantifications and activity assays were taken using a UV-Visible spectrophotometer (SHIMADZU UV-2450 spectrophotometer). Scanning electron microscope (SEM) images were taken on a HITACHI SU 8220 SEM with an energy-dispersive spectrometer (EDS). The Transmission Electron Microscope (TEM) and High-Resolution Transmission Electron Microscope (HRTEM) measurements were conducted on a FEI Tecnai G2 F20 S-TWIN microscope. Laser scanning confocal microscope images were obtained from a TCS SP8 confocal laser scanning microscopy (CLSM). X-ray diffraction (XRD) patterns were recorded using a Bruker D8 ADVANCE X-ray diffractometer at 40 kV and 40 mA. Fourier transform infrared (FTIR) spectra were measured on a Nicolet NEXUS 670 FTIR spectrometer. The nitrogen adsorption-desorption isotherm was measured at 77 K on a Micromeritics ASAP 2020 analyzer and specific surface areas were calculated using the Brunauer-Emmett-Teller (BET) equation. Fluorescence analysis was carried out on a HITACHI F-4500 fluorescence spectroscope. The circular dichroism (CD) spectra were carried out on an Applied Photophysics Chirascan dichrograph. Inductively coupled plasma atomic emission spectrometry (ICP-AES) was carried on a HITACHI Z-2000 ICP-AES.

Labelling Cyt c with FITC. 6.4 mg fluorescein isothiocyanate (FITC) was added to the Tris-HCl buffer (50 mM, 4 mL, pH 9.0) containing Cyt c (10 mg/mL). The whole solution was stirred at room temperature for 6 h in the dark. The unreacted FITC were removed by dialysis against water for one day.

Enzymatic activity assay. ABTS and H_2O_2 were used as substrates for enzymatic activity assay. The final assay solution contained ABTS (0.4 mM, 900 μL), H_2O_2 (0–100 mM) and 50 μL 332 nM Cyt c (equivalent weight of Cyt c for Cyt c@ZIF-8 and Pd-Cyt c@ZIF-8) in 50 mM Tris-HCl buffer (pH 7.4). The increase in absorbance at 405 nm was recorded for 2 min. The molar extinction coefficient of oxidized ABTS at A_{405} is 36800 M^{-1} cm^{-1}.

Kinetic parameters were obtained by measuring the initial rates of reaction with different hydrogen peroxide concentrations using a non-linear regression of the Michaelis-Menten equation.

$$V0 = \frac{V_{max}[S]}{(K_M + [S])} \quad (3)$$

where V_0 represents the initial catalytic rate, K_M represents the Michealis-Menten constant, V_{max} represents the maximum rate of conversion, [S] represents the initial concentration of substrate.

3. Results and discussion

As shown in Scheme 1, Cyt c-Pd@ZIF-8 composites were synthesized through biomimetic mineralization in an aqueous media. Biomimetic mineralization is a biologically induced process which has recently been applied for the design and fabrication of diverse organic-inorganic hybrid materials [21,22]. In a typical experiment, Pd NPs were prepared by a $NaBH_4$ reduction procedure at room temperature with PVP which served as dispersant and stabilizer [23], and Cyt c-Pd complexes were obtained by mixing Cyt c and the as-synthesized Pd NPs, then the complexes were added into the solution of ZIF-8 precursor containing zinc nitrate hexahydrate and 2-methylimidazole, finally Cyt c-Pd@ZIF-8 composites were obtained followed by incubation at room temperature for 30 min.