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Elimination of background color interference by immobilizing Prussian blue on carbon cloth: A monolithic peroxidase mimic for on-demand photometric sensing

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ABSTRACT

Prussian blue (PB) is widely used as an artificial peroxidase for electrochemical biosensors, yet it is rarely utilized in photometric detection because of its intrinsic color interference. Herein, we proposed a new strategy, growing PB on common carbon cloth (CC), to obtain a monolithic peroxidase mimic with no background interference for colorimetric detection. Through a one-pot two-step electrodeposition procedure, a robust PB film composed of compactly assembled cubes was coated on CC surface. Since the active PB is immobilized on a bulk substrate (not like previous nanozymes that were dispersed in solution), the color interference from PB itself is efficiently avoided. In addition, the obtained monolithic nanozyme can be put into and taken out from reaction systems expediently and promptly with just a tiny pair of tweezers, which makes it possible for on-demand detection. As demonstrated, the PB-based nanozyme exhibited excellent peroxidase-like activity and could trigger the color reaction of 3,3′5,5′-tetramethylbenzidine in the presence of H_2O_2, leading to a detection limit of as low as 1.7 μM for H_2O_2 sensing. A uric acid (UA) colorimetric assay was further established by incorporating the PB/CC peroxidase mimic with natural uricase, providing a linear absorbance response in the analyte concentration scope of 10–700 μM. Reliable analysis of UA in human serum and urine verified the practicability of the fabricated assay.

1. Introduction

Nanozymes are a class of nanosized materials with enzyme-like characteristics. In comparison with traditional bio-enzymes that are mostly composed of proteins, nanozymes exhibit stronger resistance against harsh environments such as extreme pH and temperature, and this distinct feature endows them with higher stability and better long-term storage [1]. Moreover, advanced nanotechnologies make it easy for large-scale preparation and adjustment of nanozymes [2], significantly reducing their production cost. With the attractive advantages mentioned above, nanozymes show great promise in chemical, analytical, environmental, and biomedical applications [3–6].

Since Gao and co-authors provided the first evidence that common ferromagnetic nanoparticles (Fe_3O_4 NPs) have intrinsic peroxidase-like activity [7], intensive attention has been rapidly focused on the exploration of new nanozymes and their various applications [1]. Currently, noble metals (Pt, Au, et al.) [8–12], non-precious transition metal (Fe, Co, Cu, Zn, Ce, et al.) derivatives [13–22], carbon-based materials (graphene, C_3N_4, et al.) [23–25], and some emerging structures (metal organic framework, layered MoS_2, et al.) [26–30] have been exploited as potential peroxidase mimics. Similar to Fe_3O_4, Prussian blue (PB) also consists of mixed valence states of Fe (Fe(III) coordinates to N at octahedral sites of the PB face-centered cubic lattice, and Fe(II) is surrounded by C). This similarity in component endows PB with high peroxidase-like catalytic activity for the reduction of H_2O_2. On this occasion, artificial PB peroxidase has been widely employed as a transducer for electrochemical biosensors [31–35]. However, it is rarely applied for photometric sensing. The main obstacle that hinders the application of PB in colorimetric analysis is its intrinsic color. The blue color of PB itself will cause strong background interference for optical measurements.

To eliminate the color interference of PB and expand its application in photometric analysis, Wang and co-workers filled PB NPs into carbon nanotubes (CNTs) to obtain a mimetic peroxidase for colorimetric sensing [36]. With the shielding of CNTs, no prominent color background of PB was observed. Similarly, Su

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et al. encapsulated PB NPs inside a metal organic framework to shield the blue color of PB [37]. Very recently, we supported PB with CNTs and obtained a mimic with high peroxidase-like activity [38]. By decreasing the amount of the nanozyme used, background color interference was also avoided successfully. Nonetheless, the above mentioned methods give rise to some shortcomings as well when they are employed to weaken or eliminate the PB color interference: the encapsulation strategy decreases reaction surface area and active sites accessible to substrates, and reducing the nanozyme amount used will prolong the catalytic reaction time. Therefore, exploring new approaches to avoid the color interference of PB-based nanozymes as well as maintaining their inherent characteristics is highly required.

Herein, we put forward a novel strategy, *in situ* immobilizing PB on carbon cloth (CC), to acquire, for the first time, a monolithic peroxidase mimic for photometric sensing. The merits of this strategy are multifold: (1) Not like previously reported nanozymes that were dispersed in solution [2], the active PB is immobilized on a bulk substrate (CC), so the background color interference from PB itself is avoided efficiently; (2) Since the prepared monolithic nanozyme is able to be put into and/or taken out from a reaction system promptly and conveniently with just tweezers (not like dispersed nanozymes where complicated centrifugation or magnetic separation was required), the catalytic reaction can be stopped and/or reinstated optionally, which makes it possible for on-demand analysis; (3) The nanozyme can be massively prepared via simple electrodeposition at low cost; (4) The active PB on CC surface is sufficiently exposed to substrates, thus exhibiting high activity and short reaction time. In the presence of H_2O_2, the prepared nanozyme could trigger the reaction of colorless 3,3′5,5′-tetramethylbenzidine (TMB) to its blue oxide (TMBox). Based on this principle, high-performance photometric detection of H_2O_2 was realized. By incorporating the PB/CC peroxidase mimic with natural uricase, a uric acid (UA) assay was further fabricated for colorimetric biosensing of the target in human serum and urine.

2. Experimental

2.1. Materials and chemicals

Conductive CC (HCP330N, 32 cm × 16 cm, 0.32 mm in thickness) was purchased from Shanghai Hesen Electric Co., Ltd. $K_3[Fe(CN)_6]$, $FeCl_3{\cdot}6H_2O$, KCl, HCl, NaOH, sodium acetate (NaAc), acetic acid (HAc), NaH_2PO_4, $Na_2HPO_4{\cdot}7H_2O$, TMB, H_2O_2, UA, ascorbic acid (AA), dopamine (DA), 4-acetamidophenol (AP), glucose (Glu), cholesterol (Chol), fructose (Fru), triglyceride (Trig), bile acid (BA) and $CaCl_2$ were obtained from Sinopharm Chemical Regent Co., Ltd. Reduced nicotinamide-adenine dinucleotid (NADH) was provided by Shanghai Aladdin Reagent Co., Ltd. Uricase (≥10 U mg^{-1}, from arthrobacter protophormiae) was provided by Shanghai Macklin Biochemical Co., Ltd. Horseradish peroxidase (HRP, ≥150 U mg^{-1}, from microorganisms) was provided by Shanghai Yuanye Biological Technology Co., Ltd. All other chemicals were of analytical grade and directly utilized without further purification. Deionized water was used in the whole study.

2.2. Preparation of the PB/CC peroxidase mimic

The PB/CC peroxidase mimic was prepared according to the literature [33] with some modifications. A CHI660E electrochemical workstation (Shanghai Chenhua Instruments Co., Ltd.) equipped with a three-electrode system consisting of a CC working electrode (4 cm × 1 cm, the area immersed in solution was 3 cm × 1 cm), a Pt wire counter electrode and an Ag/AgCl reference electrode was used for electrodeposition. The electrodeposition solution contained 2 mM $K_3[Fe(CN)_6]$, 2 mM $FeCl_3$, 0.1 M KCl and 1 mM HCl. A constant potential of 0.4 V was first applied for 120 s to form PB crystal nucleuses on CC surface. Then, cyclic voltammetric (CV) scanning in the potential window of 0.05–0.55 V at 20 mV s^{-1} was carried out in the same solution for PB film growth. After electrodeposition, the prepared PB/CC product was rinsed with adequate water and dried in an oven at 60 °C overnight for use.

2.3. Characterization

The crystal structure of the obtained product was detected by a 6100 X-ray diffractometer (XRD, Shimadzu) with Cu Kα radiation. Scanning electron microscopy (SEM) images were obtained by a JSM-6010 PLUS/LA microscope (JEOL). Transmission electron microscopy (TEM) images were obtained by a JEM-1400 microscope (JEOL).

2.4. Photometric measurements

All photometric measurements were performed on a UV–vis spectrophotometer (Cary 8454, Agilent Technologies). A 20 mM TMB stock solution was prepared with ethanol for use. H_2O_2 stock solutions with various concentrations were daily prepared. A piece of PB/CC (5 mm × 5 mm) was used as the nanozyme. After reaction in a solution system, the PB/CC nanozyme was taken out with a tiny pair of tweezers and rinsed with deionized water for three times, and then it was reused in another solution system. 0.2 M NaAc-HAc solutions with different pH values (adjusted by HCl or NaOH) were prepared as an incubation buffer. The total volume of each solution reaction system was 3 mL. Time-dependent absorbance at 652 nm was recorded with a certain time interval. Reaction kinetics was measured by recording the absorbance within 1 min at a 5 s interval. In each experimental group, only one condition (H_2O_2 or TMB concentration) varied at a time. For the photometric detection of H_2O_2, the absorbance at 652 nm was recorded after the nanozyme was put into for reaction for 5 min.

For the biosensing of UA, a certain amount of UA was added into a 0.5 mL uricase stock solution (10 U mL^{-1}, 10 mM phosphate buffer, pH 8.5) for incubation at room temperature for 10 min. After that, the solution was injected into a reaction system consisting of 0.15 mL TMB stock solution (20 mM), 2.35 mL NaAc-HAc solution (0.2 M, pH 4.0) and a piece of the PB/CC nanozyme. After reaction for 5 min, the absorbance at 652 nm was recorded.

The UA levels in human serum and urine samples were detected by the fabricated UA assay. Clinical blood and urine samples were provided by the Affiliated Hospital of Jiangsu University. Serum samples were collected by centrifugation at 3000 rpm for 5 min. For each serum sample, 300 μL of serum was added into a 0.5 mL uricase stock solution (10 U mL^{-1}, 10 mM phosphate buffer, pH 8.5) for incubation for 10 min. The reaction solution was then transferred into a reaction system composed of 0.15 mL TMB stock solution (20 mM), 2.05 mL NaAc-HAc solution (0.2 M, pH 4.0) and a piece of the PB/CC nanozyme. After reaction for 5 min, the absorbance at 652 nm was recorded. For each urine sample, a dilution of 10 times with phosphate buffer (10 mM, pH 7) was performed before measurements.

3. Results and discussion

3.1. Characterization of the PB/CC nanozyme

First, the obtained PB/CC product was characterized by XRD and SEM. As shown in Fig. 1(A), a series of well-defined diffraction peaks assigned to the PB face-centered cubic phase (PDF card no. 73-0687) are probed in the product, demonstrating that PB has been successfully immobilized on CC via the electrodeposition procedure.