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A novel nanoenzyme based on Fe_3O_4 nanoparticles@thionine-imprinted polydopamine for electrochemical biosensing

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ABSTRACT

Here, a new nanoenzyme of Fe_3O_4 nanoparticles (NPs) magnetic molecularly imprinted polymers (MMIPs) was prepared by polymerizing dopamine on the Fe_3O_4NPs surface in the presence of templated thionine (Thi) for the first time. The results showed that uniform spherical and core-shell structured Fe_3O_4NPs MMIPs which were about 600 nm in diameter were successfully formed and the imprinting sites improved the selectivity of Fe_3O_4NPs MMIPs greatly. The as-prepared Fe_3O_4NPs MMIPs could catalyze the reduction of Thi selectively, which could be enhanced by H_2O_2 owing to the peroxidase-like activity of Fe_3O_4NPs. Accordingly, a highly selective and sensitive H_2O_2 electrochemical biosensor was proposed based on the Fe_3O_4NPs MMIPs-modified glassy carbon electrode. The electrochemical biosensor based on the Fe_3O_4NPs MMIPs nanoenzyme exhibited low detection limit of 1.58 nM and high selectivity. Since acetylthiocholine chloride (AChl) could be hydrolyzed into choline with the help of acetylcholinesterase (AChE) and simultaneously the choline oxidase (ChOx) could reduce choline into betaine accompanied by the production of H_2O_2, the proposed electrochemical H_2O_2 biosensor could be further used to detect AChl, AChE and ChOx. The results also exhibited wide linear range (2.85–160 μM for AChl, 0.53–20000 ng mL^{-1} for AChE and 22.76–400 ng mL^{-1} for ChOx), low detection limit (0.86 μM for AChl, 0.16 ng mL^{-1} for AChE and 6.83 ng mL^{-1} for ChOx) and high selectivity. Therefore, the Fe_3O_4NPs MMIPs should be a promising nanoenzyme for electrochemical biosensors.

1. Introduction

The biosensors could transform the biological response into the detectable signal. Among various biosensors, the enzyme-based electrochemical biosensors have attracted extensive interests due to their convenient operation, fast signal conversion, low detection limit, and excellent specificity [1–5]. Enzyme is the protein with special catalytic function. Enzymes could be obtained from organisms by complex procedures with high cost [6–10]. Furthermore, natural enzymes are always easy to lose their bioactivity at high temperature, in a low or high pH solution, even when contact with electrode surface [11–16]. Accordingly, it is necessary to develop nanoenzymes instead of natural enzymes for constructing enzyme-based electrochemical biosensors [17–19].

Nanostructures with enzymatic activities, those are called nanoenzymes, have attracted increasing attention for natural enzyme mimics and found wide applications in bioanalysis, bioimaging, and biomedicine [20,21]. Among the known nanoenzymes, magnetic Fe_3O_4 nanoparticles (NPs) have received much attention due to their application in biological imaging and separation techniques [22–26]. In 2007, it was reported that Fe_3O_4NPs exhibited intrinsic peroxidase-like activity, just like natural horseradish peroxidase (HRP) [27]. It was well known that peroxidases have the ability to oxidize the organic substrate for reducing their toxicity which was employed for wastewater treatment. For instance, Fe_3O_4NPs could oxide 3,3′,5,5′-tetramethylbenzidine (TMB) into a blue product instead of HRP in the presence of H_2O_2 [28]. In 2014, it was also discovered that the Fe_3O_4NPs could be used as mimetic HRP to electrochemically catalyze the reduction of thionine (Thi) without H_2O_2 [29]. Although Fe_3O_4NPs is a good kind of HRP mimic enzyme, its specificity is still far lower than that of natural enzymes. This weakness has hampered the application of Fe_3O_4NPs nanoenzymes in biosensors. Therefore, to develop

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novel nanoenzymes with high specificity is still challenging and fascinating.

Molecular imprinting is a facile and well-established method to create recognition cavities in accordance with template-molecule's shape, size and functional group [30–32]. Currently, molecular imprinting polymers (MIPs) showed wide application for solid-phase extraction, catalysis, drug-controlled release, and chemical sensors due to possessing ideal selectivity, thermal stability, easy preparation, etc. [33–36]. Dopamine (DA) could be easily polymerized on a solid surface owing to its catechol and amine groups [37]. In 2012, DA was chosen as the monomer to construct bovine hemoglobin imprinted biosensors [38]. In 2013, Yao designed a magnetic MIPs (MMIPs) by DA self-polymerization on the surface of NPs in the presence of template chlorpyrifos [39].

Inspired by these breakthroughs, we designed a novel nanoenzyme of Thi-imprinted Fe_3O_4NPs MMIPs by polymerizing DA on Fe_3O_4NPs in the presence of template Thi for the first time. The Thi is an important electroactive small molecule which shows a pair of well developed redox peaks and has been extensively used as probe in electrochemical biosensors. The imprinting sites improved the selectivity of Fe_3O_4NPs MMIPs toward Thi greatly. We found the Fe_3O_4NPs MMIPs could catalyze the reduction of Thi selectively with the help of H_2O_2. Accordingly, a highly selective and sensitive electrochemical H_2O_2 biosensor was proposed based on the Fe_3O_4NPs MMIPs by using Thi as probe. Since acetylthiocholine chloride (AChl) could be hydrolyzed into choline with the help of acetylcholinesterase (AChE) and simultaneously the choline oxidase (ChOx) could reduce choline into betaine accompanied by the production of H_2O_2 [40,41], the proposed electrochemical H_2O_2 biosensor could be further used to detect AChl, AChE and ChOx, respectively.

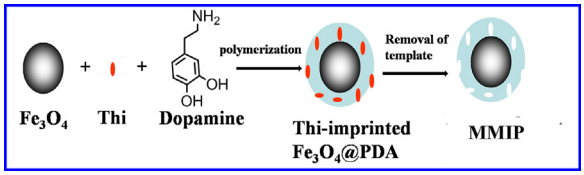
2. Experimental

2.1. Chemicals and solutions

Tris (hydroxymethyl) aminomethane (Tris), $FeCl_3{\cdot}6H_2O$, TMB, DA, Thi, AChE (type C3389, 500 U mg^{-1}), AChl, and ChOx were obtained from Sigma–Aldrich. NaAc, ethylene glycol, NaH_2PO_4, Na_2HPO_4, H_2O_2 (AR, 30 wt.% in H_2O) and other reagents were purchased from Aladdin Reagent Co., Ltd (Shanghai, China). Phosphate buffer solution (PBS, 0.2 M) was prepared by mixing Na_2HPO_4 and NaH_2PO_4. Tris-HCl buffer (pH 8.5) was prepared as followed [42]. Briefly, 12.14 g Tris were added into 1000 mL water, and then adjusted the pH to 8.5 with 6 M HCl. The ChOx, AChl and AChE solution were daily prepared by dissolving proper amount of commercial substance in 0.2 M PBS (pH 7.0) and then stored in 4 °C. Ultrapure water (18.2 MΩ cm^{-1}) was used.

2.2. Preparation of Fe_3O_4NPs MMIPs

The Fe_3O_4NPs were prepared by dissolving 0.30 g $FeCl_3$ and 0.80 g NaAc in 10 mL of ethylene glycol and then heating at 200 °C for 10 h [36]. Then 25 mg Fe_3O_4NPs powder were added into 10 mL of 10 mM Tris-HCl buffer (pH = 8.5) followed by the addition of 2.5 mL of 2.0 mg mL^{-1} Thi. Then the above suspension was mechanically stirred for 2 h. Next, 25 mg DA was dissolved in the O_2-saturated solution and incubated for 4 h. The resulted Fe_3O_4NPs MMIPs were collected by external magnetic separation. The template molecules were extracted by acetic acid/acetonitrile solution. For comparison, magnetic non-imprinted polymers (MNIPs) were also fabricated in the absence of Thi during the self-polymerization process. The preparation process was illustrated in Scheme 1.



Scheme 1. Schematic illustration of the fabrication process of Fe_3O_4NPs MMIPs.

2.3. Preparation of Fe_3O_4NPs MMIPs-modified glassy carbon electrode (GCE)

25 mg Fe_3O_4NPs MMIP powder was firstly dispersed in 5 mL of 0.05% nafion solution. Then 7 μL of 5.0 mg mL^{-1} Fe_3O_4NPs MMIPs aqueous suspension was cast on the newly polished GCE with a diameter of 3 mm from Shanghai Chenhua Instrument Co., Ltd. and then dried under N_2 atmosphere. The nafion improved the distribution and stability of Fe_3O_4NPs MMIP on GCE surface greatly. To further enhance the stability and repeatability of modified electrode, the Fe_3O_4NPs MMIPs-modified GCE was immersed in 0.2 M PBS (pH 7.0) for 4 h and then washed by ultrapure water. In the immersing process, some loosely bound Fe_3O_4NPs MMIPs could drop off from the Fe_3O_4NPs MMIPs-modified GCE, which enhanced the stability and repeatability of the Fe_3O_4NPs MMIPs-modified GCE. The MNIPs-modified GCE was also prepared by this method.

2.4. Instruments

Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDXS) tests were implemented on a HITACHI S-3400N scanning electron microscope with a Phoenix energy X-ray analyzer. X-ray powder diffraction (XRD) data were collected on a D/Max 2500V/PC X–ray powder diffractometer using Cu Kα radiation (λ = 1.54056 Å, 40 kV, 200 mA). UV–vis absorption spectra were collected on a Hitachi U-3900H UV–vis Spectrophotometer. All electrochemical measurements were performed on a CHI 660C electrochemical workstation (Shanghai, China) by conventional three-electrode system including the modified GCE as the working electrode, a platinum wire as the auxiliary electrode and a saturated calomel electrode (SCE, saturated KCl) as the reference electrode.

3. Results and discussion

3.1. Characterization of Fe_3O_4 MMIPs

The shape and core-shell nanostructure of Fe_3O_4NPs MMIPs were characterized by SEM and the results are shown in Fig. 1. The low resolution SEM image (Fig. 1A) showed a large number of uniform spherical Fe_3O_4NPs. The high resolution SEM image (Fig. 1C) indicated that the surface of Fe_3O_4NPs was very rough, which provided lots of active sites to improve its catalytic activities. The diameter of the spherical Fe_3O_4NPs was about 600 nm which was slightly bigger as compared with other nanomaterials, but similar to some previous results [36]. Although small Fe_3O_4NPs were benefit for their electrochemical performances, it was very difficult to reduce their size owing to the strong magnetism. Here, we tried our best to synthesize uniform and relatively small Fe_3O_4NPs. The good uniformity was also very beneficial to the good reproducibility of electrochemical biosensors based on Fe_3O_4NPs. The low resolution SEM image of the Fe_3O_4NPs MMIPs (Fig. 1B) also showed many uniform spherical particles but their surface seemed more smooth as compared with Fe_3O_4NPs (Fig. 1D). It might be because the DA polymerized on the surface of Fe_3O_4NPs and finally formed ploydopamine (PDA) to cover the Fe_3O_4NPs. The diameter of the Fe_3O_4NPs MMIPs was also about 600 nm, which indicated