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Self-assembled dipeptide–gold nanoparticle hybrid spheres for highly sensitive amperometric hydrogen peroxide biosensors

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ABSTRACT

Novel self-assembled dipeptide–gold nanoparticle (DP–AuNP) hybrid microspheres with a hollow structure have been prepared in aqueous solution by a simple one-step method. Diphenylalanine (FF) dipeptide was used as a precursor to form simultaneously peptide spheres and a reducing agent to reduce gold ions to gold nanoparticles in water at 60 °C. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) revealed that formed AuNPs were localized both inside and on the surface of the dipeptide spheres. Horseradish peroxidase (HRP) as a model enzyme was further immobilized on the dipeptide–AuNP hybrid spheres to construct a mediate H_2O_2 amperometric biosensor. UV–vis spectroscopy showed that the immobilized HRP retained its original structure. Cyclic voltammetry characterization demonstrated that the HRP/dipeptide–AuNP hybrid spheres modified glassy carbon electrode showed high electrocatalytic activity to H_2O_2. The proposed biosensor exhibited a wide linear response in the range from 5.0×10^{-7} to 9.7×10^{-4} M with a high sensitivity of 28.3 μA mM^{-1}. A low detection limit of 1.0×10^{-7} M was estimated at $S/N=3$. In addition, the biosensor possessed satisfactory reproducibility and long-term stability. These results indicated that the dipeptide–AuNP hybrid sphere is a promising matrix for application in the fabrication of electrochemical biosensors due to its excellent biocompatibility and good charge-transfer ability.

1. Introduction

It is existed widely in nature and well-known that some biomolecules can self-assemble into various structures (Knowles et al., 2010). Molecular self-assembly is also a powerful, bottom-up approach for fabricating novel functional nano- or biomaterial (Whitesides and Grzybowski, 2002; Ulijn and Smith, 2008). Recently, a peptide-based self-assembly is attracting increasing attention due to inherent biocompatibility, chemical versatility, biological recognition, and facile synthesis (Gazit, 2007; Hamley, 2014). Among various peptide-based building blocks, diphenylalanine (Phe – Phe, FF) and its derivatives are simplest ones that can form various nanostructures exhibiting novel optical, mechanical and electrochemical properties (Panda et al., 2008; Ryu et al., 2009; Yemini et al., 2005b). These fascinating properties make them potential as a kind of attractive and versatile materials for biosensor applications. Gazit and coworkers firstly reported that FF self-assembled peptide nanotubes (PNTs) as a novel electrochemical biosensing platform showed promising analytical performance (Yemini et al., 2005a). Thereafter PNTs were applied in the encapsulation of various biomolecules for electrochemical biosensing (Adler-Abramovich et al., 2010; Park et al., 2012; Sasso et al., 2012; Castillo et al., 2013; Baker et al., 2014). Furthermore, semiconductor quantum dots (such as CdTe and CdSe) and enzymes have been together introduced into the self-assembly of N-fluorenylmethoxycarbonyl diphenylalanine (Fmoc-FF) (Kim et al., 2011b). The optical biosensors based on the self-assembled quantum dot-peptide hydrogel composite have been successfully developed. Despite these advances, the further tuning FF-based peptide nanostructures and the introduction of more functional units into peptide entities are still highly desirable for the design of novel biosensor and the improvement of the sensing performance (de La Rica et al., 2011; Qu et al., 2014).

Noble metal nanoparticles such as gold nanoparticles (AuNPs) have been extensively employed for the preparation of inorganic–organic hybrid materials and applied in electrochemical biosensors (Zhang et al., 2010; Wang et al., 2012). Due to their excellent conductivity, good biocompatibility and high surface area, the analytical performances of the biosensors based on AuNPs hybrid systems could be significantly enhanced relative to those based on only organic components. Compared with general organic compounds, the biological molecules, especially for peptides, have unique advantages in preparing well-designed AuNP hybrid

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nanostructures because of their abundant constitutes, specific chelate ability to inorganic ions and easy control of the crystal structure (Dickerson et al., 2008; Chen and Rosi, 2010; Song et al., 2010). However, the studies on the combination of AuNPs and FF self-assembled nanostructures are very limited (Yan et al., 2010a). To the best of our knowledge, no other group has reported the functionalization of dipeptide spheres with AuNPs for the immobilization of enzyme to construct electrochemical biosensor.

Herein, considering the above mentioned advantages of self-assembled dipeptide nanostructures and AuNPs, novel dipeptide–AuNP hybrid spheres with hollow structure were synthesized through a simple self-assembly process. Such composites were further employed to immobilizing a model enzyme, horseradish peroxidase (HRP), for the construction of an electrochemical H_2O_2 biosensor. Hydroquinone (HQ) was chosen as an electron-mediator (Camacho et al., 2009; Won et al., 2010). Electrochemical measurements were conducted to investigate the catalytic performance of the proposed biosensor. The good properties of the biosensor based on the dipeptide–AuNP hybrid spheres were demonstrated.

2. Experimental section

2.1. Materials

Diphenylalanine (FF) peptide in a lyophilized form was obtained from Bachem (Bubendorf, Switzerland). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was purchased from Aladdin Chemicals Co. Ltd., China. The $HAuCl_4 \cdot 4H_2O$ (99.9%) was purchased from Sinopharm Chemical Reagent Co. Ltd., China. Horseradish peroxidase (HRP, RZ ≥ 3, activity ≥ 250 units mg^{-1}) was obtained from Shanghai Xueman Biotechnology Co. Ltd. (China). Hydroquinone (HQ) was purchased from Tianjin Fuchen Chemical Reagent Works (China). Hydrogen peroxide (H_2O_2, 30%) was obtained from Beijing Chemical Works (China). Nafion (5 wt%) was purchased from Sigma-Aldrich. The solutions used for electrochemical characterization were freshly prepared using 0.1 M of phosphate buffer solution (PBS, pH 7.0) unless otherwise noted. All other reagents were of analytical grade and used without further purification. All solutions were prepared with deionized, doubly distilled water (DDW).

2.2. Preparation of dipeptide–AuNP Hybrid spheres

A FF stock solution was freshly prepared by dissolving the lyophilized FF in HFIP at a concentration of 100 mg mL^{-1}. The stock solution was then diluted with 0.5 mM $HAuCl_4$ solution to a final concentration of 2 mg mL^{-1}. The mixture solution was stirred for 30 min, followed by incubation at 60 °C in the dark for 1.5 h. The color of the solution changed from light yellow to light purple. The suspension of the dipeptide–AuNP hybrid spheres was prepared. Herein, the synthesized conditions have been optimized to obtain the hybrid spheres with good morphology and uniform structure according to the concentration of peptide and $HAuCl_4$, as well as synthesized temperature and time.

2.3. Preparation of HRP/dipeptide–AuNP hybrid sphere modified electrodes

Prior to use, glassy carbon electrodes (GCE, diameter 3 mm) were first polished with 1.0, 0.3, and 0.05 μm alumina slurry sequentially, followed by rinsing with DDW, sonicating and then drying by nitrogen. Enzyme electrode was fabricated by a simple casting method. An aliquot (6 μL) of the dipeptide–AuNP hybrid sphere suspension was dropped on the pretreated GCE and left to dry at room temperature. Then 2 mg mL^{-1} of HRP solution (6 μL) was dropped on the dipeptide–AuNP hybrid sphere modified GCE and dried at 4 °C overnight in a humidity chamber. The electrode was then coated with 3 μL of 0.5 wt% Nafion solution and dried at 4 °C. Finally the modified electrode was washed gently with DDW three times to remove unbounded enzyme, denoted as a HRP/DP–AuNP/GCE. In control experiment, a HRP/GCE without dipeptide–AuNP hybrid spheres was also fabricated using the same procedure. The as-prepared electrodes were stored at 4 °C when not in use.

2.4. Instruments and measurements

Scanning electron microscopy (SEM) images were recorded with a Zeiss Supra 55 field emission scanning electron microscope equipped with energy-dispersive X-ray spectroscopy (EDS). Transmission electron microscopy (TEM) images were obtained on a Hitachi H-800 electron microscope with an accelerating voltage of 200 kV. For the SEM and TEM measurements, the suspension of the dipeptide–AuNP hybrid sphere was dropped onto a silicon wafer and a copper grid, respectively, followed by drying at room temperature. UV–vis absorbance spectra were recorded on a UV–vis spectrophotometer (Perkin-Elmer Lambda 35).

Electrochemical experiments were conducted on a CHI 660B electrochemical workstation (Shanghai CH Instruments, China). A conventional three-electrode system was employed with a modified glassy carbon electrode as the working electrode, a platinum wire as the counter electrode, and a saturated Ag/AgCl electrode as the reference electrode. The cyclic voltammetric measurements were carried out in an unstirred electrochemical cell. Amperometric curves were obtained by consequently adding H_2O_2 solution with a certain concentration into PBS containing 1.0 mM HQ at -0.05 V after achieving a steady state current. The working solutions were deoxygenated with nitrogen gas for 15 min before measurements and a nitrogen atmosphere was kept over the solutions throughout the experiments.

3. Results and discussion

3.1. Morphological characterization and formation mechanism of the dipeptide–AuNP hybrid spheres

The strategy employed to prepare the dipeptide–AuNP hybrid spheres and the HRP-based biosensor was illustrated in Scheme 1. Firstly, the FF/HFIP monomer solution was added into the $HAuCl_4$ solution. After 60 °C for 1.5 h, the color of the solution changed from light yellow to light purple, indicating that the gold ions were reduced to form AuNPs (Tan et al., 2010; Kim et al., 2011a). The obtained solution was characterized in detail by SEM and TEM, as shown in Fig. 1. The sub-micrometer peptide spheres with embedded AuNPs were observed from the SEM image (Fig. 1A). The average diameter of the hybrid spheres and AuNPs was about 812 nm and 27 nm, respectively. EDS analysis (Fig. 1B) confirmed that the elemental composition of the hybrid spheres. TEM images (Fig. 1C and D) further showed that the AuNPs were not only localized on the surface of the dipeptide spheres, but also embedded inside the hybrid spheres (see Fig. S1). Furthermore, the partially aggregated AuNPs and the hollow structure in the hybrid spheres were also observed from TEM images. For the fabrication of the modified electrode, the obtained dipeptide–AuNP hybrid spheres were further casted onto the GCE surface, and then the HRP solution was also casted onto the surface of the hybrid sphere modified GCE. Finally, they were enclosed to the electrode surface by coating Nafion membrane as protective additive, which is widely used in the preparation of electrochemical biosensors (Wang et al., 2010; Park et al., 2012; Baker et al., 2014).