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Electrochemical immunosensor based on Pd–Au nanoparticles supported on functionalized PDDA-MWCNT nanocomposites for aflatoxin B1 detection

Songbai Zhang [a, b], Youming Shen [a], Guangyu Shen [a, *], Sha Wang [a], Guoli Shen [b], Ruqin Yu [b]

[a] Department of Chemistry and Chemical Engineering, Hunan University of Arts and Science, Changde 415000, People's Republic of China
[b] State Key Laboratory for Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, People's Republic of China

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ABSTRACT

This paper reports a label-free electrochemical immunosensor for the determination of aflatoxin B1 (AFB1), which is based on a gold electrode modified by a biocompatible film of carbon nanotubes/poly(diallyldimethylammoniumchloride)/Pd–Au nanoparticles (CNTs/PDDA/Pd–Au). The nanocomposite was characterized by transmission electron microscopy and the electrochemical behavior of modified electrodes was investigated by cyclic voltammetry. The CNTs/PDDA/Pd–Au nanocomposites film showed good electron transfer ability, which ensured high sensitivity to detect AFB1 in a range from 0.05 to 25 ng mL^{-1} with a detection limit of 0.03 ng mL^{-1} obtained at 3σ (where σ is the standard deviation of the blank solution, $n = 10$). The proposed immunosensor provides a simple tool for AFB1 detection. This strategy can be extended to any other antigen detection by using the corresponding antibodies.

Introduction

Aflatoxins are a group of fungal mycotoxins produced by *Aspergillus flavus* and *A. parasiticus* [1]. Among twenty kinds of aflatoxins, aflatoxin B1 (AFB1) is the most toxic, carcinogenic, mutagenic, and genotoxic [2]. It is found in a variety of contaminated or moldy crops and beverages such as corn, peanuts, nuts, almonds, figs, milk and other foods. Several techniques for AFB1 determination, including high-performance liquid chromatography (HPLC), enzyme-link immunosorbent assay (ELISA), and thin-layer chromatography (TLC), have been developed [3–5]. Although these methods are sensitive and accurate, they are time-consuming, require expensive equipment and materials, and need tedious washing steps. To overcome these disadvantages, various electrochemical biosensors have been developed for AFB1 detection. For example, an electrochemical quartz crystal microbalance for the quantitative detection of AFB1 in groundnuts has been developed [6]. Highly sensitive impedimetric immunosensors for AFB1 detection have also been reported [7–10]. Although these methods are low-cost and relatively easy to use, their sensitivity needs to be further improved. Additionally, the detection of AFB1 has been achieved by amperometric biosensors [11]. Nevertheless, there is still increasing demand for novel immunosensors for AFB1 detection.

Carbon nanotubes (CNTs) have attracted considerable interest because of their high electrical conductivity, chemical stability, high surface area, and mechanical strength [12,13]. They are used not only as a substrate material to improve the electron transport of sensing interfaces [14,15], but also as a carrier of signal materials in sandwich-type immunsensors [16] to amplify electrochemical signals. The preparation of CNT modified electrodes is usually carried out by dropping CNT solution onto the electrode surface and then drying in air. But CNTs are easy to aggregate through strong π–π stacking and van der Waals interaction [17], resulting in a discontinuous and nonuniform film. It is reported that the aggregation of CNTs can be prevented by ionic liquids through cation–π interactions between ionic liquids and CNTs [18]. However, ionic liquids are small molecules and water-soluble; they can leak out from substrates, which results in poor repeatability of biosensors. Recently, poly(diallyldimethylammoniumchloride) (PDDA), a linear

* Corresponding author.
E-mail address: sgyrab@163.com (G. Shen).

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positively charged polyelectrolyte, has attracted great attention as a means to improve the dispersion in aqueous solutions of carbon nanomaterials such as graphite [19], CNTs [20], and carbon nanofibers [21]. In addition, PDDA easily forms standing thin films on the electrode surface and interacts well with oppositely charged nanoparticles [22,23], thus making it suitable for the simple fabrication of biosensors.

Nanocomposites of metal nanoparticles and CNTs have high electrocatalytic activity toward various bioelectrochemical reactions [24,25]. But most metal nanoparticles will not adhere to hydrophobic carbon nanotubes [26]. To improve metal deposition onto CNTs, surface modification of the CNTs has been carried out. For example, CNTs modified by positively charged Azure can adsorb negatively charged gold nanoparticles via electrostatic interaction [27]. Furthermore, bimetallic nanoparticles are better catalysts and electrocatalysts than to the separate components [28,29]. This characteristic offers a promising prospective for developing biosensors [30,31].

In this work, a label-free electrochemical immunosensor for the detection of AFB1 was developed based on CNTs/PDDA/Pd–Au nanocomposites. PDDA was used not only to improve the dispersion of CNTs in aqueous solutions, but also to enrich positive charges on the surfaces of CNTs for adsorbing Pd–Au nanoparticles. The presence of CNTs warrants fast electron transfer, and Pd–Au nanoparticles provide a good substrate for antibody immobilization. The fabricated immunosensor showed good sensitivity, selectivity, and reproducibility and could be used for the detection of AFB1 in real samples with satisfactory results.

Experimental

Reagents and apparatus

AFB1 was obtained from Beijing Lianlixin BioTech Co., Ltd. Mouse anti-AFB1 monoclonal antibody was purchased from Beijing Hapten and Protein Biomedical Institute. Bovine serum albumin (BSA) and human serum albumin (HSA) were obtained from Beijing Dingguo Biotechnology Company (Beijing, China). A 0.1 M phosphate buffer solution (PBS, pH 7.0) was prepared using Na_2HPO_4 and NaH_2PO_4. Multiwall carbon nanotubes functionalized by –COOH (CNTs) (95% purity, diameter 20–30 nm) were purchased from Nanjing Xianfeng Nanotechnology Co. (Nanjing, China). Poly(diallyldimethylammonium chloride) (PDDA, Mw = 200,000–350,000) in 20% aqueous solution, $HAuCl_4$, K_2PdCl_4, and ascorbic acid were purchased from Sigma–Aldrich.

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were carried out with a CHI 660 electrochemistry workstation (Shanghai CH Instruments, China) in 10 mM $K_3Fe(CN)_6$/$K_4Fe(CN)_6$ solution (5 mM of each complex). A conventional three-electrode system was used, including a Pt electrode as counter electrode, a saturated calomel electrode (SCE) as reference electrode, and a gold electrode modified with CNTs/PDDA/Pd–Au as working electrode. CV measurements were taken at a scanning rate of 100 mVs^{-1} from −0.2–0.6 V relative to the saturated calomel electrode.

Preparation of Pd–Au nanoparticles

The Pd–Au nanoparticles were synthesized by the method reported by Lee et al. with a slight modification [32]. Typically, 1 mL of a 5 mM aqueous solution of an $HAuCl_4$/K_2PdCl_4 mixture (molar ratios of 1:1) was added to 47 mL of purified water. Then ascorbic acid (100 mM, 50 mL) was added to the mixed solution. After 15 s, an aqueous solution of polyvinyl pyrrolidone (5 mg mL^{-1}, 1 mL) was added dropwise with vigorous stirring. After addition, the mixture was stirred at 25 °C for another 1 h and then transferred into 1 mL centrifugal tubes. The obtained Pd–Au nanoparticles were collected by centrifugation at 12,000 rpm for 10 min and washed three times with ultrapure water. Then the product was redispersed into 0.5 mL water for further use.

Preparation of CNTs/PDDA/Pd–Au nanocomposites

CNTs/PDDA/Pd–Au nanocomposites were prepared according to the reported method with a slight modification [33] and the process is shown in Fig. 1A. Briefly, 1.0 mg acid-treated CNTs was dispersed into 1 mL of a 0.25 wt.% PDDA aqueous solution containing 0.5 M NaCl and ultrasonicated for 1 h to obtain a homogeneous black suspension. The resulting dispersion was centrifuged and washed with water three times to remove redundant PDDA. The collected product CNTs/PDDA was redispersed in 1 mL water and the resulting solution sonicated for 10 min before use. Then the CNTs/PDDA suspension was mixed with two times concentrated Pd–Au nanoparticles (1 mL) and stirred for 1 h. A CNTs/PDDA/Pd–Au nanocomposite was obtained, which was sonicated for 10 min before the films were prepared.

Fabrication of the immunosensor

The bare Au electrode (3 mm diameter) was first polished with emery paper and alumina slurry of 0.3 and 0.05 μm, followed by successive sonication in distilled water and ethanol. The electrode was then treated with fresh piranha solution (1:3, v/v, H_2O_2 and H_2SO_4) for 3 min and washed with distilled water. Then the Au electrode was subjected to electrochemical cleaning by scanning over a potential range from −+0.3 to +1.5 V in freshly prepared 0.5 M H_2SO_4 until a voltammogram characteristic of a clean gold electrode was established. A sample of 10 μL of CNTs/PDDA/Pd–Au solution was dropped on the electrode surface, followed by evaporation of the solvent in air. A CNTs/PDDA/Pd–Au film-modified Au electrode (CNTs/PDDA/Pd–Au/Au) was obtained. After washing with water, 10 μL of 150 μg mL^{-1} antibody was added onto the electrode to be incubated for 40 min at 37 °C. Then the electrode modified with antibody was incubated with 10 μL BSA (2.0 wt.%) for 40 min at 37 °C to eliminate nonspecific binding. Finally, 10 μL of antigen solution at a specific concentration was added to the electrode to be incubated for 30 min at 37 °C, followed by washing with distilled water prior to electrochemical measurements. The whole process of immunosensor fabrication is shown in Fig. 1B.

Sample preparation

The sample preparation was carried out according to the method reported by Tan [34]. Noncontaminated rice obtained from a local market was first ground. Aliquots (1 g) of ground rice were spiked with AFB1 at different concentrations and mixed in a vortex mixer. After the addition of 5 mL of extraction solvent (80% methanol), the samples were mixed by shaking for 45 min and then centrifuged at 5000 rpm for 10 min. The supernatant was carefully removed and diluted with PBS (1:5, v/v) for AFB1 detection.

Results and discussion

CNTs/PDDA/Pd–Au nanocomposites

The preparation of CNTs/PDDA/Pd–Au nanocomposites was carried out in two steps. First, the PDDA was bound to the carboxyl-functionalized CNT surfaces via hydrophobic and electrostatic interaction to form a stable CNTs/PDDA composite. Second, positively charged CNTs/PDDA nanocomposite was mixed with