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Dumbbell-like Au-Fe_3O_4 nanoparticles as label for the preparation of electrochemical immunosensors

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ABSTRACT

In this work, we developed a novel kind of label based on dumbbell-like Au-Fe_3O_4 nanoparticles (NPs) for the preparation of electrochemical immunosensor for the detection of cancer biomarker prostate specific antigen (PSA). The signal amplification strategy, using the synergetic effect present in Au-Fe_3O_4 to increase the reduction ability of the NPs toward H_2O_2, improves the sensitivity and detection limit of the immunosensor. With primary anti-PSA antibody (Ab_1) immobilized onto graphene surface and secondary anti-PSA antibody (Ab_2) adsorbed onto the Au of the Au-Fe_3O_4 NPs, the immunosensor prepared through a sandwich structure displays a wide linear range (0.01–10 ng/mL), low detection limit (5 pg/mL), good reproducibility and stability. These labels for immunosensors may provide many potential applications for the detection of different biomolecules.

1. Introduction

Due to the highly specific molecular recognition of immunoreaction, immunosensors based on antibody–antigen binding have found wide applications in different fields including environmental monitoring, food safety and clinical diagnosis (Wu et al., 2007; Liu and Ju, 2005; Rusling et al., 2009; Tang et al., 2008). Electrochemical immunosensors offer a number of significant advantages, including high sensitivity, low cost, and easy of miniaturization when compared with several other types of immunosensors based on fluorescence, chemiluminescence, surface-plasmon resonance, or quartz crystal microbalance (Cui et al., 2007; Fan et al., 2005; Papkovsky et al., 1999; Yuan et al., 2001; Zhang et al., 2007). Hence, different kinds of electrochemical immunosensors, especially amperometric immunosensors, have gained growing interests and developed into one of the major analytical technique for the detection of biomolecules.

To increase the sensitivity of the immunosensor, various types of nanomaterials have been investigated as labels, including metal nanoparticles (NPs), quantum dots, carbon nanotubes, and electroactive component-loaded nanoparticles (Lai et al., 2009; Wu et al., 2009; Darain et al., 2003; Liu and Ju, 2008). Ho et al. reported using quantum dot cadmium sulfide to label secondary antibody, and with the primary antibody immobilized on carbon nanoparticle surface, the immunosensor can be used to detect carcinoembryonic antigen (CEA) as low as 32 pg/mL (Ho et al., 2009). Tang et al. demonstrated using thionine-doped magnetic gold nanospheres as labels, and with enzyme horseradish peroxidase adsorbed onto the nanospheres as enhancer, the immunosensor displayed a detection limit of 5 pg/mL toward CEA (Tang and Ren, 2008).

Since the discovery of carbon nanotubes in 1991, carbon nanomaterials have attracted considerable attention recently because of their remarkable conductivities and appealing electrochemical properties. Recently, as one kind of carbon nanostructure, graphene sheet (GS) has stimulated a vast amount of research due to its fascinating properties (Geim and Novoselov, 2007; Ohta et al., 2006; Aleiner and Efetov, 2006). It has large specific surface area, extraordinary flexibility and electronic transport property. GS is considered as the basic building block that can be wrapped into 0D fullerenes, rolled into 1D carbon nanotube and stacked into 3D graphite. Therefore, GS can be potentially used as a new sort of electrochemical material. For example, Zhou et al. reported electrochemical sensing and biosensing platform based on graphene (Zhou et al., 2009).

Metal NPs dispersed on an oxide support often display much higher catalytic activity than single component NPs, which is due to synergetic effect that occurs at the interface of metal and oxide support (Valden et al., 1998; Wang et al., 2009a; Zheng and Stucky,

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2006; Comotti et al., 2006; Liu et al., 2006; Lee et al., 2010a). Recent studies indicate that Au NPs are normally chemically inert, but Au NPs deposited on a metal-oxide support have shown high catalytic activity for CO oxidation (Chen and Goodman, 2004). Wang et al. reported Pt NPs in the Pt-Fe_3O_4 structure shows a 20-fold increase in mass activity toward oxygen reduction reaction compared with the single component Pt NPs (Wang et al., 2009b).

In this work, the dumbbell-like Au-Fe_3O_4 NPs were prepared and indeed showed synergetic effect in catalyzing H_2O_2 reduction, which is more active than Au or Fe_3O_4 alone. With the secondary antibody (Ab_2) adsorbed onto Au, the resulting Au-Fe_3O_4-Ab_2 were used as label for the preparation of immunosensor. Prostate specific antigen was used as model, and the sandwich-type structure is formed by immobilizing the primary anti-PSA antibody (Ab_1) onto GS surface, the PSA in the sample captured, and Au-Fe_3O_4-Ab_2 as label. The large surface area of GS can increase Ab_1 loading, and the good conductivity of GS can also help the H_2O_2 detection (Du et al., 2010). This simple, economic and sensitive immunosensing approach could find wide potential application in clinical analysis.

2. Materials and methods

2.1. Apparatus and reagents

Graphite was purchased from Shanghai Carbon Co., Ltd. (Shanghai, China). Prostate specific antigen (PSA) and anti-PSA antibody were purchased from Dingguo Biochemical Reagents (Beijing, China). $HAuCl_4{\cdot}H_2O$, tert-butylamine–borane (TBAB, 97%), 1,2,3,4-tetrahydronaphthalene (tetralin, 99%) and 1-octadecene, $[Fe(CO)_5]$ (98%) were obtained from Sigma–Aldrich. Oleylamine and oleic acid were got from Acros Organics. All other chemicals were of analytical reagents grade and used without further purification. Phosphate buffered saline (PBS, 0.1 M containing 0.1 M NaCl, pH 7.4) was used as electrolyte for all electrochemistry measurement. Double distilled water was used throughout the experiments.

All electrochemical measurements were performed on a CHI 760D electrochemical workstation (Shanghai CH Instruments Co., China). Transmission electron microscope (TEM) images were obtained from a JEOL JEM-2010 microscope (Japan). A conventional three-electrode system was used for all electrochemical measurements: a glassy carbon electrode (GC, 3 mm in diameter) as the working electrode, an Ag/AgCl electrode as the reference electrode, and a platinum wire electrode as the counter electrode.

2.2. Preparation of graphene sheet

GS was prepared from graphite oxide (GO) through a thermal exfoliation method (McAllister et al., 2007). At first, GO powders were produced from graphite by a modification of Hummer's method (Liu et al., 2008). In a typical experiment, 5 g of graphite was oxidized by reacting with 100 mL of concentrated H_2SO_4 under stirring for 12 h. Then, while immersing the reaction vessel in an ice bath, 30 g of $KMnO_4$ was added slowly. After the addition of $KMnO_4$, the solution was stirred at 100 °C for another 12 h to fully oxidize graphite GO. The obtained GO was then thoroughly washed and dried. Thermal exfoliation of GO was achieved by placing GO (100 mg) into a quartz tube under argon atmosphere. The quartz tube was flushed with argon for 10 min, and then quickly inserted into a furnace preheated to 1000 °C and held in the furnace for about 1 min.

2.3. Preparation of dumbbell-like Fe_3O_4 nanoparticles

To prepare Au-Fe_3O_4, first, 6 nm Au seed was prepared. Typically, a solution of tetralin (10 mL), oleylamine (10 mL), and $HAuCl_4{\cdot}3H_2O$ (0.1 g) was prepared at room temperature (20 °C) and initially stirred for 10 min. TBAB (1 mmol), tetralin (1 mL), and oleylamine (1 mL) were mixed by sonication and quickly injected into the above solution. The reaction mixture was further stirred at room temperature for 1 h. Au seeds were precipitated by ethanol addition, washed with hexane and ethanol, and collected by centrifugation.

The Au-Fe_3O_4 was prepared according to literature report (Lee et al., 2010a). The pre-synthesized 6 nm Au seed in hexane (1 mL) were added to a solution of 20 mL of 1-octadecene with oleic acid (1 mL) and oleylamine (1 mL). The mixture was heated up to 120 °C under a gentle N_2 flow to remove hexane. Under a N_2 blanket, $[Fe(CO)_5]$ (0.1 mL) was injected into the solution. The solution was heated to reflux (300 °C) and left at that temperature for 30 min. Thereafter, it was cooled down to room temperature and was exposed to air to form Au-Fe_3O_4 nanoparticles. Isopropanol was added to precipitate the Au-Fe_3O_4 NPs, which were washed extensively with hexane, ethanol and PBS and then collected by centrifugation.

2.4. Preparation of Au-Fe_3O_4-Ab_2 conjugation

Fig. 1A shows the procedure to prepare the Au-Fe_3O_4-Ab_2 conjugate. The as synthesized Au-Fe_3O_4 NPs in PBS (2 mg/mL) were added to into anti-PSA Ab_2 solution (1 mL, 100 μg/mL) and the mixture was stirred for 12 h. After centrifuge and washing with PBS, the Au-Fe_3O_4-Ab_2 conjugation was re-dispersed in PBS and stored at 4 °C before use.

2.5. Modification of electrodes

Primary anti-PSA Ab_1 was immobilized onto the GS surface through an amidation reaction between the carboxylic acid groups on GS and the available amine groups of Ab_1. Into GS solution (2 mg/mL), EDC and NHS (100 mM) were added. The mixture was stirred for 4 h and after that, 50 μg/mL of Ab_1 solution was added into the mixture. After another 12 h of reaction, the GS solution was centrifuged and washed. The resulting GS-Ab_1 conjugates were stored at 4 °C in phosphate buffer solution before use.

The fabrication procedure of the immunosensor is shown in Fig. 1B. GC electrode was polished with 1, 0.3, and 0.05 μm alumina powder sequentially and then washed ultrasonically in ethanol and water for a few minutes, respectively. Onto electrode surface, 5 μL of the GS-Ab_1 mixture was added and dried. After washing, the electrode was then incubated in 1% BSA solution for another 30 min to block nonspecific binding sites. Following that, the electrodes were incubated with different concentrations of PSA solution for

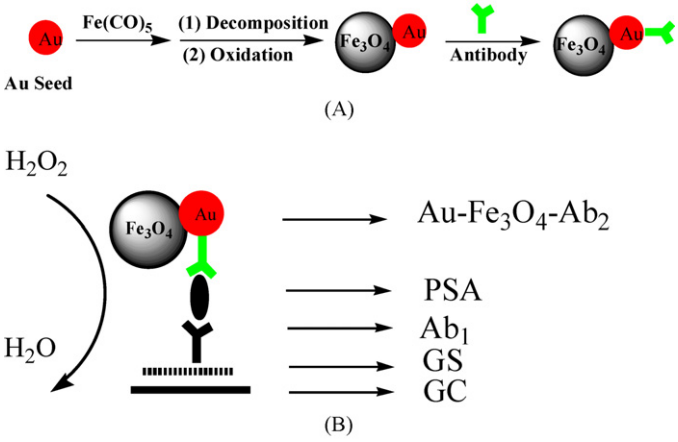

Fig. 1. Schematic representation of the preparation of the Au-Fe_3O_4-Ab_2 nanostructure (A) and immunosensor (B).