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Au–ionic liquid functionalized reduced graphene oxide immunosensing platform for simultaneous electrochemical detection of multiple analytes

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ABSTRACT

In this work, an Au–ionic liquid functionalized reduced graphene oxide nanocomposite (IL-rGO–Au) was fabricated via the self-assembly of ionic liquid functionalized reduced graphene oxide (IL-rGO) and gold nanoparticles (AuNPs) by electrostatic interaction. The IL-rGO can be synthesized and stabilized by introducing the cations of the amine-terminated ionic liquids (IL-NH_2) into the graphene oxide (GO). With the assistance of IL-NH_2, AuNPs were uniformly and densely absorbed on the surfaces of the IL-rGO. The proposed IL-rGO–Au nanocomposite can be used as an immunosensing platform because it can not only facilitate the electrons transfer of the electrode surface but also provide a large accessible surface area for the immobilization of abundant antibody. To assess the performance of the IL-rGO–Au nanocomposite, a sandwich-type electrochemical immunosensor was designed for simultaneous multianalyte detection (carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) as model analytes). The chitosan (CS) coated prussian blue nanoparticles (PBNPs) or cadmium hexacyanoferrate nanoparticles (CdNPs) and loaded with AuNPs were used as distinguishable signal tags. The resulting immunosensor exhibited high selectivity and sensitivity in simultaneous determination of CEA and AFP in a single run. The linear ranges were from 0.01 to 100 ng mL^{-1} for both CEA and AFP. The detection limits reached 0.01 ng mL^{-1} for CEA and 0.006 ng mL^{-1} for AFP, respectively. No obvious nonspecific adsorption and cross-talk was observed during a series of analyses to detect target analytes. In addition, for the detection of clinical serum samples, it is well consistent with the data determined by the ELISA, indicating that the immunosensor provides a possible application for the simultaneous multianalyte determination of CEA and AFP in clinical diagnostics.

1. Introduction

In clinical diagnosis, simultaneous multianalyte immunoassays are promising analytical methods for their intrinsic advantages such as short analytical time, simplified analytical procedure, decreased sampling volume, improved test efficiency and reduced cost compared with parallel single analyte assays (Li et al., 2012; Hansen et al., 2006). Among the different techniques for simultaneous multianalyte immunoassays, electrochemical technique has its special merit: high sensitivity; inherent simplicity; and low cost (Tang et al., 2013; Kong et al., 2013; Lai et al., 2011; Lin et al., 2011; Peng et al., 2009; Wang, et al., 2013, 2012; Shi and Ma, 2011). Despite some advances in this field, it is still a challenge to explore new strategies for further improvement of the simplicity, sensitivity and accuracy.

Graphene-nanoparticle composites have become a hot research topic in material science because the composite process can be an effective strategy to enhance their electronic, chemical, and electrochemical properties (Xiao et al., 2012; Feng et al., 2012, 2011). Generally, there are two approaches to load nanoparticles on graphene: (1) *in situ* synthesized in the presence of graphene (Han et al., 2013; Li et al., 2009); and (2) adsorbed nanoparticles on graphene from their colloidal dispersions (Gao et al., 2013; Liu et al., 2013). In electrochemical application, flexible control over the size, morphology, degree of loading, and distribution of the nanoparticles on the graphene is critical for optimizing the performance of the electrodes (Fang et al., 2010). However, the first approach may lead to hardly control both the size and coverage density of nanoparticles on graphene. The serious problem associated with the second approach is the aggregation of reduced graphene nanosheets, implying that it is very hard to obtain completely dispersed single graphene-nanoparticle composites (He and Gao, 2010).

To resolve such problems, here we show a ionic liquid functionalized reduced graphene oxide loaded with gold nanoparticle

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nanocomposite (IL-rGO–Au) that integrates both the electrically conductivity property of graphene, hydrophilicity of ionic liquid and large specific surface area and active sites to immobilize antibody of gold nanoparticles (AuNPs). Compared with the routine approaches, our work shows two advantages. First, the cations of the amine-terminated ionic liquid (IL-NH_2) can be introduced into the graphene oxide (GO), contributing to stabilization of ionic liquid functionalized reduced graphene oxide (IL-rGO) dispersions via electrostatic repulsion. Second, the surfaces of IL-rGO are evenly covered with uniform AuNPs, and the AuNPs deposited on IL-rGO are noticeably dense.

Another important issue for the successful development of multiplexed immunoassays is to search distinguishable redox probes as trace labels. Generally, an ideal of multiple tags for protein label should meet several requirements. One is based on each target on the identical sensing interface to achieve the independent response, and another is based on distinguishable voltammetric signals to avoid interaction with analytes and the sample matrix (Han et al., 2012). Prussian blue and cadmium hexacyanoferrate belong to a group of excellent electron-transfer mediators that have attracted enormous attention. As a result, the prussian blue nanoparticles (PBNPs) and cadmium hexacyanoferrate nanoparticles (CdNPs) have received increasing attention in the field of biosensors because of the large surface-to-volume ratio, high electronic catalytic activity and the easy controllable size (Baioni et al., 2007). However, PBNPs or CdNPs modified biosensors usually show poor stability and short lifetime as their water solubility (Hong et al., 2008). Here, we choose chitosan (CS) to coat PBNPs (or CdNPs) to enhance the stability of PBNPs (or CdNPs) as well as functionalize the interface of PBNPs (or CdNPs) via the amino-group of CS. Thus, AuNPs were modified on the surface of CS-coated PBNPs (or CdNPs) to form a three layer PB–CS-Au (or Cd–CS-Au) nanoparticle, which was used for the preparation of distinguishable signal tags.

In this work, IL-rGO–Au nanocomposite was fabricated via the self-assembly of IL-rGO and AuNPs by electrostatic interaction. The proposed IL-rGO–Au nanocomposite can be used as an immunosensing platform because it exhibited excellent electrically conductivity property, hydrophilicity and large specific surface area. Inspired by these promising properties of the IL-rGO–Au, a simple and sensitive electrochemical immunoassay was designed for simultaneous multianalyte detection (carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) as model analytes) using the IL-rGO–Au as matrix, PB–CS-Au and Cd–CS-Au as distinguishable signal tags. The electrochemical signals were simultaneously obtained at two peak potentials, because of the presence of PBNPs and CdNPs. With the sandwich-type immunoassay format, the antigen–antibody immunocomplex was formed on the surface of the IL-rGO–Au. The peak currents and the peak position were dependent on the concentration and type of the corresponding analytes, respectively.

2. Experimental

2.1. Materials

IL-NH_2 (e.g., 1-aminopropyl-3-methylimidazolium chloride) was from Shanghai Chengjie Chemical Co. Ltd. (Shanghai, China). Mouse anti human monoclonal antibody to carcinoembryonic antigen (anti-CEA) (Isotype: IgG_1; Source: Mouse Ascites), mouse anti human alpha fetoprotein monoclonal antibody (anti-AFP) (Isotype: IgG_1; Source: Mouse Ascites), CEA (Source: Human colon cancer) and AFP (Source: Human fetal cord serum) and were purchased from Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). Human immunoglobulin G (IgG) (Isotype: IgG; Source: Normal Human Serum) was purchased from Chengwen Biological Company (Beijing, China). GO was obtained from JCNANO (Nanjing, China). Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot xH_2O$), ferric trichloride ($FeCl_3$), cadmium chloride ($CdCl_2$), D-(+)-glucose, ascorbic acid (AA), uric acid (UA) and sodium borohydride ($NaBH_4$) were achieved from Alfa Aesar (Tianjin, China). Clinical human serum samples were provided by the Capital Normal University Hospital (Beijing, China). Trisodium citrate, NaH_2PO_4, Na_2HPO_4, KCl, KOH, C_2H_5OH, H_2O_2, H_2SO_4, albumin from bovine serum (BSA), potassium ferricyanide ($K_3Fe(CN)_6$), and potassium ferrocyanide ($K_4Fe(CN)_6$) were obtained from Beijing Chemical Reagents Company (Beijing, China). All the reagents were of analytical grade and used as received.

2.2. Apparatus

In all the procedures, the water used was purified through an Olst ultrapure K8 apparatus (Olst, Ltd., resistivity > 18 MΩ). Transmission electron microscopy (TEM) was performed with a JEOL-100CX electron microscope under 80 kV accelerating voltage. X-ray photoelectron spectroscopy (XPS) was conducted using an Escalab 250 X-ray Photoelectron Spectroscope (Thermofisher, American) employing a monochromatic Al Kα radiation. Electrochemical measurements were carried out on CHI-832 electrochemical workstation (Chenhua Instruments Co., Shanghai, China). A three-electrode system was used in the experiment with a glassy carbon electrode (GCE) (4 mm in diameter) as the working electrode, an Ag/AgCl electrode (saturated KCl) and a Pt wire electrode as reference electrode and counter-electrode, respectively.

2.3. Synthesis of AuNPs

AuNPs with the sizes of 3.5, 5, 10, 15, 20, 25 and 30 nm used in this work were synthesized according to the as-reported method (Sun and Ma, 2012). The 3.5 nm AuNPs were prepared at room temperature by adding 1 mL 1% sodium citrate solution to the 100 mL 0.01% $HAuCl_4$ solution with stirring. After 1 min, 2 mL 0.075% $NaBH_4$ (dissolved in 1% sodium citrate solution) was added. The mixture was not stopped stirring until its color turned red. About 5 and 10 nm AuNPs, the preparing procedures were the same as that of 3.5 nm except 1.6 or 1 mL $NaBH_4$ solution was added. For 15, 20, 25 and 30 nm AuNPs, they were obtained by adding 5, 4, 3.5 and 3 mL 1% sodium citrate solution, respectively, to 100 mL 0.01% boiling $HAuCl_4$ solution with vigorous stirring. The color changed from pale to blue, then to burgundy. Boiling was continued for 15 min and then stirring until the sample had cooled to room temperature.

2.4. Preparation of IL-rGO–Au nanocomposite

IL-rGO was synthesized according to the literature with a little modification (Yang et al., 2009). Briefly, 10 g IL-NH_2 was added into 50 mL of GO homogeneous dispersion in water (0.5 mg mL^{-1}), and then the salt effect of the GO occurred, owing to the presence of ionic liquid. Then, 50 mg KOH was added into the above turbid mixture and then the mixture was subjected to ultrasonication for 30 min. After the ultrasonication, the turbid mixture was transformed into a homogeneous solution. Finally, the homogeneous solution was vigorously stirred at 80 °C for 24 h. The resulting IL-rGO was subsequently centrifuged, washed with ethanol and ultrapure water, and dispersed in ultrapure water (1 mg mL^{-1}).

IL-rGO–Au nanocomposite was synthesized by simply blending in an appropriate ratio of IL-rGO and AuNPs for above 2 h. Then, the resulting solution was centrifuged at 6000 rpm, and then washed and dispersed in ultrapure water (1 mg mL^{-1}).