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Electrochemiluminescence of luminol enhanced by the synergetic catalysis of hemin and silver nanoparticles for sensitive protein detection

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ABSTRACT

A novel and ultrasensitive electrochemiluminescence (ECL) immunosensor, which was based on the amplifying ECL of luminol by hemin-reduced graphene oxide (hemin-rGO) and Ag nanoparticles (AgNPs) decorated reduced graphene oxide (Ag-rGO), was constructed for the detection of carcinoembryonic antigen (CEA). For this proposed sandwich-type ECL immunosensor, Au nanoparticles electrodeposited (DpAu) onto hemin-rGO (DpAu/hemin-rGO) constructed the base of the immunosensor. DpAu had outstanding electrical conductivity to promote the electron transfer at the electrode interface and had good biocompatibility to load large amounts of primary antibody (Ab_1), which provided an excellent platform for this immunosensor. Moreover, AgNPs and glucose oxidase (GOD) functionalized graphene labeled secondary antibody (Ag-rGO–Ab_2–GOD) was designed as the signal probe for the sandwiched immunosensor. Not only did the hemin-rGO improve the electron transfer of the electrode surface, but hemin also further amplified the ECL signal of luminol in the presence of hydrogen peroxide (H_2O_2). With the aid of Ag-rGO–Ab_2–GOD, enhanced signal was obtained by in situ generation of H_2O_2 and catalysis of AgNPs to ECL reaction of the luminol–H_2O_2 system. The as-prepared ECL immunosensor exhibited excellent analytical property for the detection of CEA in the range from 0.1 pg mL^{-1} to 160 ng mL^{-1} with a detection limit of 0.03 pg mL^{-1} ($SN^{-1}=3$).

1. Introduction

Recently, electrochemiluminescence (ECL) has become an extremely attractive method because of its simplified set-up, low background signal, high sensitivity, rapidity and controllability (Jiang and Ju, 2007; Cao et al., 2006; Jie et al., 2010). As one of the most commonly used efficient ECL luminophores, luminol has some excellent inherent properties such as inexpensive, nontoxic and high light-emitting quantum yield (Qin et al., 1998; Lin and Chen, 2006), so special attention had focused on the ECL studies concerning luminol for food testing (Haghighi and Bozorgzadeh, 2011; Guo et al., 2011b), clinical diagnostics (Lv et al., 2004; Shen et al., 2011) and DNA analysis (Zhang et al., 2009; Chai et al., 2010). In order to amplify the ECL signal of luminol, several efforts have been devoted to accelerate the oxidization of luminol, through hastening the decomposition of H_2O_2 to form reactive oxygen species (ROSs) such as superoxide anion $O_2^{\bullet -}$ and hydroxyl radical $OH^{\bullet}$, because these free radicals are the important intermediates leading to the ECL reaction (Vitt et al., 1991; Cai et al., 2010). To the best of our knowledge, nanoparticles, which have virtues of good biocompatibility, large surface area, excellent electrocatalytic activity and fascinating conductivity, not only facilitate the immobilization of enzyme and proteins but also catalyze H_2O_2 to generate a great amount of ROSs. For instance, our group reported an ECL immunosensor based on the luminol–H_2O_2 system using Pd nanoparticles as catalysis for ultrasensitive immunoassay (Niu et al., 2011). Cheng et al. (2012) developed an ultrasensitive luminol ECL immunosensor for CEA detection based on ZnO nanoparticles. Among the wide variety of metal nanoparticles, AgNPs show excellent catalytic and electrocatalytic activities (Zhang et al., 2005; Jana et al., 1999). In addition, Guo et al. (2008) had demonstrated that AgNPs exhibited better catalysis activity than gold and platinum nanoparticles. Therefore, inspired by the superior property, AgNPs can be selected as an efficient catalyst in ECL sensors for its low oxidation potential when oxidized by H_2O_2 (Guo et al., 2008).

Hemin, with high electrocatalytic activity due to its reversible redox potential of Fe^{III}/Fe^{II}, can catalyze a variety of oxidation reactions like peroxidase enzymes (Genfa and Dasgupta, 1992). However, direct application of hemin as an oxidation catalyst is of

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a significant challenge because of its molecular aggregation, which causes passivation of its catalytic activity (Bruice, 1991). In order to improve the stability or activity, the hemin decorated on materials with high surface area is becoming an alternative approach. For example, G-quadruplex DNA oligomers, graphene or carbon nanotube have been considered as excellent carriers for hemin (Jiang et al., 2013; Yuan et al., 2012; Xie et al., 2012; Guo et al., 2011a, 2011b; Tu et al., 2010; Liu et al., 2007). Based on the functionalized hemin, many electrochemical (Wang et al., 2013; Bai et al., 2011) or chemiluminescence (CL) (Pavlov et al., 2004; Liu et al., 2011) sensors have been successfully constructed and exciting results have been obtained. However, until now, researchers have concentrated little attention on the study of the application of hemin in the ECL sensor.

In the present research, we constructed a novel and simple sandwich-type immunosensor for CEA detection via amplifying the ECL signal of luminol by the catalysis of hemin-rGO and Ag-rGO. In brief, DpAu obtained by electrodeposition on the hemin-rGO nanocomposites was served as the immunosensor platform, which played two main roles. On one hand, DpAu amplified the ECL signal of luminol for its fascinating conductivity, on the other hand, DpAu served as carriers to immobilize primary antibody (Ab_1) for its favorable biocompatibility. Moreover, AgNPs-rGO nanocomposites were used to load secondary antibody (Ab_2) and GOD. In the presence of oxygen, these loaded GOD immediately catalyzed the oxidation of glucose in the detection to in situ generate H_2O_2, which could subsequently promote the oxidation of luminol with an amplified ECL signal. Additionally, hemin and AgNPs could further enhance the ECL signal of luminol owing to the decomposable catalysis of H_2O_2 to produce increased amounts of ROSs. With excellent sensitivity, selectivity and stability, the as-proposed ECL immunosensor based on hemin-rGO and AgNPs-rGO provided great potential in clinical applications.

2. Experimental

2.1. Reagents and material

Graphene oxide (GO) was obtained from Nanjing Xianfeng Nano Co. (Nanjing, China). Hemin, luminol (98%), glucose oxidase (GOD), $AgNO_3$ and bovine serum albumin (BSA, 96–99%) were bought from Sigma-Aldrich Co. (St. Louis, MO, USA). Gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$) and sodium borohydride ($NaBH_4$) were purchased from Sinopharm Chemical Reagent Co. Ltd. (China). Carcinoembryonic antibody (Anti-CEA), carcinoembryonic antigen (CEA), fetoprotein antigen (AFP), prostate specific antigen (PSA) and human chorionic gonadotropin (HCG) standard solutions were obtained from Biocell (Zhengzhou, China). The serum samples were provided by South-west Hospital (Chongqing, China). 1×10^{-2} M Stock solution of luminol was prepared by dissolving luminol in 0.1 M NaOH solution and storing it at 4 °C when not in use. Phosphated buffered solution (PBS) (pH 7.4, 0.1 M) was prepared using 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4 and 0.1 M KCl. The standard CEA, AFP, PSA and HCG stock solutions were prepared with PBS (pH 7.4) and stored at 4 °C. All other chemicals and solvents used were of analytical grade and were used as received. Double-distilled water was used throughout this study.

2.2. Apparatus

The ECL signals were monitored with a model MPI-A ECL analyzer (Xi'an Remax Electronic Science & Technology Co. Ltd., Xi'an, China) with the voltage of the photomultiplier tube (PMT) set at 800 V in the process of detection and the potential scan from 0.2 to 0.7 V. Cyclic voltammetric (CV) measurements and depositions were carried out on a CHI 610A electrochemistry workstation (Shanghai CH Instruments, China). All experiments were performed with a conventional three-electrode system containing a platinum wire as counter electrode, an Ag/AgCl (sat. KCl) as reference electrode and the modified glassy carbon electrode (GCE Φ=4 mm) as working electrode. The scanning electron micrograph was taken by using scanning electron microscopy (SEM, S-4800, Hitachi, Japan). UV–vis absorption spectra were performed on a Lambda 17 UV–vis 8500 spectrometer (PE Co., USA) added to a quartz cuvette. Transmission electron microscope (TEM) images were acquired by a transmission electron microscope (TEM, TECNAI 10, Philips Fei Co., Hillsboro, OR, Japan).

2.3. The preparation of hemin-rGO and Ag-rGO nanocomposites

Hemin-rGO nanocomposites were synthesized with a simple wet-chemical strategy through the π–π interactions by following the procedure according to the literature (Zhang et al., 2012) with a minor modification. Briefly, 20.0 mL of the homogeneous graphene oxide dispersion (0.5 mg mL^{-1}) was mixed with 20.0 mL of hemin aqueous solution (0.5 mg mL^{-1}) and 200.0 μL of ammonia solution. Then 30 μL hydrazine solution was added into the resulting mixture. After vigorously stirring for a few minutes, the vial was put in a water bath (60 °C) for 3.5 h. Next, the product was obtained by filtration and washed several times with double-distilled water. The obtained hemin-rGO nanocomposites can be redispersed readily in water by ultrasonication. Moreover, the preparation of rGO was similar to that of hemin-rGO without the addition of hemin.

The Ag-rGO nanocomposites were prepared according to the reference (Li and Liu, 2010) with a slight modification by the reduction of silver ions in the GO dispersion solution. First, 2 mL $AgNO_3$ solution (0.01 M) was added to 20 mL GO solution (0.25 mg mL^{-1}) with stirring for 30 min. Subsequently, an alkaline solution of sodium borohydride (10 mL, 0.8 mg mL^{-1}) was added to the resulting homogeneous solution. Then the mixture was vigorously stirred at room temperature for another 2 h. Finally, the color of the dispersion solution changed from brown to black. Then the product was collected by centrifugation and washed several times with double-distilled water. The obtained compounds were stored in the refrigerator at 4 °C when not in use. Additionally, the preparation of Pt-rGO and Pd-rGO nanocomposites were similar to that of Ag-rGO.

2.4. Preparation of GOD and Ab_2 labeled Ag-rGO (Ag-rGO–Ab_2–GOD) bioconjugate

The Ag-rGO–Ab_2–GOD bioconjugates were prepared according to the following steps. Briefly, 1 mL of the above Ag-rGO suspension was mixed with 0.2 mL anti-CEA, allowing them to react under soft stirring at 4 °C for 6 h. Then, Ag-rGO–Ab_2 bioconjuagtes were obtained by centrifugation at 10,000 rpm for 15 min at 4 °C to remove excess anti-CEA. Subsequently, 1 mL GOD (1 mg mL^{-1}) was added into the obtained Ag-rGO–Ab_2 bioconjuagtes and incubated for 4 h at 4 °C to block the remaining active sites of the AgNPs surface. At last, the Ag-rGO–Ab_2–GOD bioconjugates were collected by centrifugation and redispersed in 1 mL PBS (pH 7.4) and stored at 4 °C until use.

2.5. Fabrication of the ECL immunosensor

Scheme 1 shows the schematic illustration of the ECL immunosensor fabrication process. The glassy carbon electrode (GCE,