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Electrochemical immunoassay based on gold nanoparticles and reduced graphene oxide functionalized carbon ionic liquid electrode

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ABSTRACT

In this paper, a gold nanoparticle, reduced graphene oxide (R-GO) and poly(L-Arginine) composite material modified carbon ionic liquid electrode (CILE) was used as the platform for the construction of a new electrochemical carcinoembryonic antigen (CEA) immunosensor. The poly(L-Arginine)/R-GO composite film was used to modify CILE to fabricate Arg/R-GO/CILE through electropolymerization of L-Arginine on R-GO/CILE. Gold nanoparticles (AuNPs) were adsorbed on the modified electrode to immobilize the CEA antibody and to construct the immunosensor. The stepwise assembly process of the immunosensor was characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). By combining the specific properties such as the biocompatibility and big surface area of AuNPs, and the excellent electron transfer ability of R-GO and the high conductivity of CILE, the synergistic effects of composite increased the amounts of CEA antibody adsorbed on the electrode surface and then resulted in the great increase of the electrochemical responses. Under the optimal conditions, differential pulse voltammetric responses of $[Fe(CN)_6]^{3-/4-}$ were proportional to CEA concentration in the range from 0.5 to 200 ng mL^{-1} with the detection limit as 0.03 ng mL^{-1} (S/N = 3). The proposed immunosensor showed good reproducibility, selectivity, and acceptable stability.

1. Introduction

Increasing attention has been focused on the development of immunoassay because it has become a major analytical tool in clinical diagnosis [1]. In immunoassay, the determination of cancer markers associated with certain tumors in patients plays an important role in diagnosing cancer diseases. Carcinoembryonic antigen (CEA), an acidic glycoprotein with a molecular weight of about 200 kDa, is one of the most extensively used tumor markers. The normal range for CEA in an adult non-smoker is <2.5 ng/mL and for a smoker is <5.0 ng/mL. An elevated CEA level in serum may be an early indication of lung cancer, ovarian carcinoma, colon cancer, breast cancer and cystadenocarcinoma [2]. Hence, developing rapid, simple and sensitive immunoassay methods for measuring serum CEA concentration has great clinical significance in the diagnosis of cancer. However, sensitive detection of protein biomarkers remains a great challenge as CEA present at ultralow levels in the early state of diseases. It is very important to explore a new method for signal amplification in order to increase the sensitivity of the detection. Different methods for signal enhancement have been investigated, such as enzyme labeling [3], rolling circle amplification [4] and nanomaterial labeling [5]. Among these methods, nanomaterial labeling has gained growing interest due to the intrinsic advantages of nanomaterials, such as low cost, good thermal stability and large surface area [6,7].

Reduced graphene oxide (R-GO) is a sheet of sp^2 bonded two-dimensional carbon atoms that are arranged into a honeycomb structure, which has attracted considerable attentions due to its unique and excellent properties, such as extremely high thermal conductivity, good mechanical strength, high mobility of charge carriers, high specific surface area, quantum hall effect and upstanding electric conductivity [8–10]. As electrode materials, R-GO can be used for promoting electron transfer between the electroactive species and the electrode and provide a novel method for fabricating chemical sensors or biosensors [11–13]. Recently, the composite materials combining R-GO and polymer have received increased attention due to the synergistic contribution of two or more functional components and the many potential applications [14,15]. On the other hand, gold nanoparticles (AuNPs) have been widely used for the construction of electrochemical immunosensors [16,17] due to the fact that they can increase the amount of the biomolecules loaded and then amplify the response.

Ionic liquids (ILs), which are composed of organic cations and various anions, have been widely used in the fields of chemistry due to the unique advantages such as high chemical and thermal stability,

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negligible vapor pressure, high ionic conductivity, wide electrochemical windows and low toxicity [18]. Carbon ionic liquid electrode (CILE) has showed the advantages including high electronic conductivity, remarkable electrocatalytic activity, inexpensive reagents and easy fabrication. For example, Sun et al. applied the CILE as the basal electrode for the redox protein electrochemistry with different nanoparticles such as $CaCO_3$ nanoparticles [19] and CdS nanorods [20].

In this work, we proposed a novel electrochemical immunosensor for CEA based on the advantages of R-GO, AuNPs and poly(L-Arginine). A CILE was fabricated by the addition of 1-butylpyridinium hexafluorophosphate ($BPPF_6$) in carbon paste as binder and modifier, and further used as the basal electrode for the electrochemical CEA immunosensor. The AuNPs prepared by one-step direct chemical reduction were used to immobilize the CEA antibody (anti-CEA). The interaction between anti-CEA and antigen was investigated by the electrochemical probe of ferricyanide. Enhanced sensitivity was achieved by using the large specific surface area of AuNPs to increase anti-CEA loading, the high conductivity of R-GO, CILE and Au nanoparticles to promote electron transfer among probe and the electrode, which resulted in the high sensitivity of the immunosensor. Based on signal amplification strategy of R-GO and CILE, the fabricated immunosensors using AuNPs as labels showed a linear response within the wide range of 0.5–200 ng mL^{-1} of CEA, low detection limit, good reproducibility and selectivity, as well as acceptable stability.

2. Experimental

2.1. Materials

1-Butylpyridinium hexafluorophosphate ($BPPF_6$, >99%, Lanzhou Greenchem ILS, LICP, CAS, China), graphite powder was obtained from Shanghai Chemical Reagent Corporation (Shanghai, China). $HAuCl_4 \cdot 4H_2O$ was bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium citrate was purchased from Beijing Chemical Reagent Company (Beijing, China). L-Arg was purchased from ZhongBei LinGe Biotechnology Ltd. (Beijing, China). Bovine serum albumin (BSA), prostate-specific antigen (PSA), low density lipoprotein (LDL) and human immunoglobulin (HIgG) were obtained from Sigma (Saint Louis, MO, USA). CEA and anti-CEA were purchased from Biocell (Zhengzhou, China). Phosphate-buffered saline (PBS, 0.01 M) with various pH values was prepared with stock standard solution of Na_2HPO_4, NaH_2PO_4 and 0.1 M KCl. All other reagents were used without any further purification. All the solutions were prepared with doubly-distilled water.

2.2. Instruments

All the voltammetric measurements were performed on a CHI 660D electrochemical workstation (Shanghai CH Instrument, China). A three-electrode system was employed for the electrochemical detection, which was composed of a modified CILE as working electrode, a Pt wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. The pH measurements were made with a pH meter (MP 230, Mettler-Toledo, Greiffensee, Switzerland). The images of scanning electron microscope (SEM) were obtained at Hitachi S-4800 (Japan).

2.3. Preparation of R-GO nanocomposite

Graphene oxide was firstly synthesized from graphite according to the Hummers and Offeman method [21]. Then the graphene oxide was reduced and followed a typical procedure: the resulting graphene oxide dispersion (100 mL) was mixed with 70 μL of hydrazine solution (50 wt.% in water) and 0.7 mL of ammonia solution (28 wt.% in water). The mixture was stirred for 1 h at the temperature of 95 °C. Finally, black hydrophobic R-GO sheets were obtained by filtration and dried in vacuum.

2.4. Preparation of AuNPs

AuNPs were prepared by a trisodium citrate reduction method as reported before [22]. Briefly, trisodium citrate (5 mL, 38.8 mM) was rapidly added to a boiling solution of $HAuCl_4$ (50 mL, 1 mM), and the solution was kept continually boiling for another 30 min to give a wine-red solution. After filtering the solution through a 0.45-μm Millipore syringe to remove the precipitate, the filtrate was stored in a refrigerator at 4 °C.

2.5. Fabrication of the immunosensor

Carbon ionic liquid electrode (CILE) was fabricated based on the reported procedure [23]. 3.0 g of graphite powder and 1.0 g of $BPPF_6$ were mixed thoroughly in a mortar and further heated at 80 °C to form a homogeneous carbon paste. A portion of the carbon paste was filled into one end of a glass tube ($\Phi = 3$ mm) and a copper wire was inserted through the opposite end to establish an electrical contact. The CILE surface was smoothed on a piece of weighing paper just before use.

0.1 mg R-GO was dispersed into 1 mL DMF to form 0.1 mg mL^{-1} R-GO dispersion. Then 10 μL R-GO dispersion was dropped onto the surface of the CILE, dried under the infrared lamp, and finally rinsed with water to remove loosely adsorbed R-GO. Thus, the R-GO/CILE electrode was obtained.

The poly(L-Arg) film was electropolymerized on R-GO/CILE by dipping the R-GO/CILE into PBS (pH 6.0) containing 2.0 mM L-Arg with cyclic voltammetric sweeps in the potential range from −2.0 to 2.5 V at 100 mV s^{-1} for 10 cycles [24]. Then poly(L-Arg) films could be obtained at the surface of R-GO/CILE. The prepared Arg/R-GO/CILE was cleaned with water and dried under a stream of nitrogen.

The AuNPs/Arg/R-GO/CILE was prepared by immersing the Arg/R-GO/CILE into the AuNP solution for 6 h and then cleaned with water and dried under a stream of nitrogen. The AuNPs were adsorbed onto the Arg/R-GO/CILE by chemisorption type interactions between the NH_2 group and AuNPs [25]. Then the modified electrode (AuNPs/Arg/R-GO/CILE) was immersed in the anti-CEA solution at 4 °C overnight. At last the resulting electrode was incubated in BSA solution (0.25%, w/w) for about 1 h in order to block possible remaining active sites and avoid the non-specific adsorption. The finished immunosensor (anti-CEA/AuNPs/Arg/R-GO/CILE) was stored at 4 °C when not in use.

2.6. Experimental measurements

The electrochemical characteristics of the electrode were characterized by cyclic voltammetry. After the immunoreaction was performed by immersing the immunosensor in 0.01 M PBS (pH 7.0) containing various concentrations of CEA for 15 min at 30 °C and then washed carefully with double distilled water, the electrochemical measures were performed in an unstirred electrochemical cell. The CV measurements were taken from 0 to 0.7 V (vs. SCE) at 50 mV s^{-1} in 10 mL 0.01 M PBS (pH = 7.0). Electrochemical impedance spectroscopy measurements were carried out in the presence of a 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture as a redox probe in 0.01 M PBS (containing 0.1 M KCl, pH 7.0). The alternative voltage is 5 mV and the frequency range is 0.1 to 100,000 Hz. The detection is based on the oxidation peak current response decreasing after antigen–antibody reaction.