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Sandwich-like electrochemiluminescence aptasensor based on dual quenching effect from hemin-graphene nanosheet and enzymatic biocatalytic precipitation for sensitive detection of carcinoembryonic antigen

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ABSTRACT

A new and simple sandwich-like electrochemiluminescence (ECL) aptasensor for carcinoembryonic antigen (CEA) assay was fabricated based on the dual quenching effect from hemin-graphene (H-rGO) nanosheet and enzymatic biocatalytic precipitation (BCP) on the Au-CdS nanocomposites-based ECL system. In this aptasensor platform, flower-like spherical Au-CdS nanocomposites were used as ECL luminophores and exhibit a strong ECL signal. The rGO nanosheet was used as a supporter to immobilize hemin molecules via π-π stacking interactions. Due to the steric hindrance and quenching effect of rGO, the ECL intensity decreased by the construction of the sandwich "CEA aptamer I (NH_2-DNA)-CEA-aptamer II" (H-rGO-aptamer II) mode. In the process of BCP, the ECL intensity further decreased because the hemin with intrinsic peroxidase-like catalytic activity could oxidize the 4-chloro-1-naphthol (4-CN) to produce an insoluble precipitation on the sensor. Using this dual quenching strategy, the prepared aptasensor exhibits a linear range from 0.8 pg/mL to 4 ng/mL and a detection limit of 0.28 pg/mL. This ECL aptasensor has simple design and undemanding in operation and was utilized to determine the content of CEA in complex samples with recoveries of 95.0% to 115.8%. Moreover, no any chemical modification of aptamer was required, suggesting that the proposed ECL aptasensor could be applied for the detection of diverse proteins just by altering the aptamer sequence.

1. Introduction

The increasing demand of determination of disease-related proteins, especially cancer biomarkers, has received more and more attention in many fields. Carcinoembryonic antigen (CEA), a type of glycoprotein generated by tumor cells, was widely used as a tumor marker [1]. The specific and sensitive determination of CEA has great significance for clinical diagnosis and treatment assessment of cancers. Thus, many analytical methods for CEA detection have been studied, such as amperometric assay [2], fluorescence analysis [3], electrochemical method [4], photoelectrochemical assay [5], enzyme-linked immunosorbent assays [6] and capillary electrophoresis [7]. Among these methods, antibodies were mainly acted as recognition elements. However, the limits of antibodies are easy deactivation and instability. Aptamer, a kind of new recognition element, shows high bind affinity to its target and possesses numerous remarkable advantages such as design flexibility, easy synthesis and specificity [8]. So far, aptameric-based protein analytical systems have been greatly developed. For instance, in Guo's group [9], two different kinds of aptamers (with amidogen and thiol group) specific to CEA were modified on the surface of Ru@SiO_2 and Au NPs based on localized surface plasmon resonance for CEA detection. Wu et al. [10] constructed an aptasensor for CEA detection based on fluorescence resonance energy transfer, an amino group modified CEA aptamer was covalently tagged on the PAA-UCPs which was employed as the energy donor. Noticed that the aptamers for protein detection were all chemically modified, which not only limited the association with the number of target binding sites, but also increased the operation and expenses. In addition, many literatures reported that graphene sheet can be served as a supporter to adsorb ss-DNA due to its high surface area and π-π conjunction [11]. Therefore, attaching aptamer onto the graphene sheet as a probe may be an alternative for constructing a sensor with good property.

Electrochemiluminescence (ECL) approach is competitive with conventional assays because of its high sensitivity, rapid response and low background [12,13]. In recent years, various semiconductor

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nanomaterials with ECL activity have been growing [14]. CdS nanomaterials are the most promising and effective materials, owing to their excellent luminescent properties in the presence of coreactant. According to reports, Au nanoparticles exhibited a strong surface plasmon resonance effect on the CdS because Au can increase the light absorption and enhance the charge separation [15–17]. Wang et al. [15] reported an obvious enhancement of photocatalytic activity in the CdS-Au-CdS nanorod arrays, which was relative to that of pure CdS because surface plasmon resonance effect of Au segments increased the effective optical length inside the CdS. Therefore, depositing AuNPs on the spherical CdS is also expected to obtain enhanced intensity compared with the pure CdS.

Hemin, the active center of heme-protein, has the peroxidase-like activity similar to the peroxidase enzyme. In general, hemin could intercalate into G_4 structure to form G_4H DNAzyme with high peroxidase-like catalytic activity and there are no disadvantages of the natural enzymes such as instability, long-time consumption. In our previous work [18], hemin-DNAzyme with high electrocatalytic ability was used to quench ECL signal of MoS_2-CdS nanocomposites for sensitive detection of IgE. Nevertheless, it is difficult for hemin to maintain high catalytic activity in aqueous solution due to its low solubility and easy aggregation [19]. In order to solve this problem, various nanomaterials have been used as nanocarriers to load hemin, such as ZnO [20], metal-Organic frameworks [21], supramolecular hydrogels [22]. Graphene [23], with large surface area and good biocompatibility, has been also served as a valuable candidate for immobilization hemin. In addition, graphene possesses a rich surface chemistry and has the potential to promote the catalytic activity and stability of the supported molecular systems by cation-π interactions or π-π stacking. Duan's group [24] reported that hemin attached on the graphene still retained the catalytic-active monomer form as in natural enzymes. So far, H-rGO with peroxidase activity has been widely applied in many aspects, such as distinguishing between ss- and ds-DNA [25], electrochemical and ECL sensor [26,27]. Tao and co-workers [28] described a sensing strategy by employing ss-DNA probe and H-rGO sheets to detect a wide range of targets including metal ions, small molecules and DNA. A label-free colorimetric method for PDGF-BB and thrombin assay on the basis of H-rGO-DNA composite was reported by Zhang's group [29]. However, as far as we know, little attention has been paid to the applications of the above-mentioned rGO-based hybrid peroxidase mimetics in ECL aptasensor.

Except for the nanomaterial-based labeling strategy, employing enzymatic reaction to form insoluble product on the sensing interface, e.g. enzymatic biocatalytic precipitation (BCP), is an important concern for obtaining low limits of detection. Tang et al. [30] utilized the formed hemin-based DNAzyme concatamers toward catalytic precipitation of 4-CN for recognizing Cu^{2+}. Our group [31] prepared a competitive ECL aptasensor by introducing BCP technique for the sensitive detection of IgE.

Herein, we fabricated a new and simple sandwich-like ECL aptasensor for sensitive CEA assay by coupling with H-rGO-aptamer II composite as probe. The probe was used as both quencher and catalyst of BCP. The monodisperse CdS nanoparticles were synthetized via a hydrothermal method and used as a support to load high amounts of AuNPs to prepare flower-like spherical Au-CdS nanocomposite. H-rGO-aptamer II composite could use as an excellent platform for assembling ss-DNA containing a 24-base tail and a 19-base CEA aptamer sequence. In the presence of CEA, aptamers specifically combined with CEA to form a sandwich structure with the 24-base fragment still adsorbed on rGO. The ECL intensity was quenched due to the steric hindrance and quenching effect of rGO. Simultaneously, in view of the intrinsic peroxidase-like activity of hemin catalyzed to produce insoluble product on the electrode after incubated with 4-CN, further quenched the ECL signal. Under the optimum conditions, the aptasensor for CEA assay was well established and exhibits good performance to determine CEA in human serum samples.

2. Experimental

2.1. Materials and reagents

Carcinoembryonic antigen (CEA) aptamers 5′-NH_2-$(CH_2)_6$-TTT TAT ACC AGC TTA TTC AAT T-3′ (aptamer I, NH_2-DNA) and 5′-CCC ATA GGG AAG TGG GGG ATG TGT GTG TGT GTG TGT GTG TGT G (aptamer II) were synthesized by Sangon biotech Co. Ltd. (Shanghai, China). CEA was purchased from Zhengzhou Immuno Biotech Co., Ltd. (Zhengzhou, China). Hemin was from Sigma-Aldrich Chemical Co. (St. Louis, MO). Cadmium acetate [$Cd(CH_3COO)_2 \cdot 4H_2O$] and thiourea were got from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Sodium L-glutamate monohydrate was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). $HAuCl_4 \cdot 3H_2O$ and glutaraldehyde (GA) were acquired from Alfa Aesar (Tianjin, China) and Tianjin Yongda Chemical Reagent Development Center (Tianjin, China), respectively. Human IgG (hIgG), human serum albumin (HSA), and bovine serum albumin (BSA) were purchased from Shanghai Solarbio Bioscience & Technology Co., Ltd. (see bio Biotechnology). 4-chloro-1-naphthol (4-CN) was from Shanghai Ziyi Reagent Company (Shanghai, China). Ethylene glycol, Ammonia (28%) and hydrazine hydrate (35%) were from Aladdin industrial corporation (Shanghai, China). Phosphate-buffered saline (PBS) solutions with various pHs were prepared by mixing different volumes of NaH_2PO_4 and K_2HPO_4 containing 0.1 M KCl as the supporting electrolyte. Ultrapure water (Kangning Water Treatment Solution Provider, Chengdu, China) was used throughout the experiments.

2.2. Apparatus

The ECL emissions were recorded using a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) with a CR 120 type photomultiplier tube (Binsong Photonics, Beijing, China). All ECL experiments were performed with a conventional three-electrode system comprised of a Pt wire as the counter electrode, an Ag/AgCl electrode as reference electrode and bare or modified glass carbon electrodes (GCE, $\varphi = 3$ mm) as the working electrodes. Electrochemical impedance spectroscopy (EIS) was performed using an RST5200F electrochemical workstation (Zhengzhou Shiruisi Technology Co., Ltd., Zhengzhou, China) in the solution of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) containing 0.1 M KCl. Scanning electron microscopy (SEM) images and transmission electron microscope (TEM) images were acquired using a S-4800 (Hitachi, Tokyo, Japan) and Tecnai G^2 F20 TEM (FEI Co., Hillsboro, Oregon, USA), respectively. X-ray photoelectron spectra (XPS) analysis was performed on a K-Alpha X-ray photoelectron spectrometer (Thermo Fisher Scientific Co., USA). UV–visible detection was carried out using an UV mini-1240 UV–vis spectrophotometer (Shimadzu Corp., Kyoto, Japan).

2.3. Synthesis of Au-CdS nanocomposites

2.3.1. Synthesis of spherical CdS nanoparticles

The monodisperse CdS nanoparticles were synthesized following a typical procedure [32]. Firstly, 2.4 mmol $Cd(CH_3COO)_2$ was dissolved in 60 mL water and sonicated for a few minutes. Then 24 mmol thiourea was added in the above solution after vigorous stirring for 30 min, the mixture was transferred to a Tefion-lined autoclave (100 mL) and heated to 200 °C for 5 h. After naturally cooled, the precipitate was washed with water and ethanol for three times, respectively, and dried at 60 °C.

2.3.2. Synthesis of Au-CdS nanocomposites

Au-CdS nanocomposites were prepared as previously described [33]. Briefly, 0.55 mL $HAuCl_4$ aqueous solution was added into 50 mL of ethylene glycol, followed by the dissolution of 55 mg glutamate into the above solution with vigorous stirring. The pH was then adjusted to 11 by NaOH and 53 mg of CdS nanomaterial was subsequently slowly