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Photoelectrochemical immunoassay based on chemiluminescence as internal excited light source

Shenguang Ge, Linlin Liang, Feifei Lan, Yan Zhang, Yanhu Wang, Mei Yan, Jinghua Yu*

School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, PR China

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ABSTRACT

The hybrids, *N*-aminobutyl-*N*-ethylisoluminol (ABEI), horseradish peroxidase (HRP) and cancer antigen 125 s antibody (Ab2) immobilized graphene oxide (GO), were prepared, which exhibited excellent chemiluminescence (CL) activity. The zinc oxide nanorods (ZNRs) grew on reduced graphene oxide (RGO) modified paper working electrode (ZNRs-RGO/PWE) via an in situ growth method. The deposition of CdS quantum dots (QDs) on ZNRs/RGO-PWE resulted in an enhanced excitation and photo-to-electric conversion efficiency. The ABEI-GO@HRP/Ab2CL system was employed as an internal excited light source. A photoelectrochemical (PEC) immunosensor based on CdS-sensitized ZNRs anchored RGO as the photoactive matrix and ABEI-GO@HRP/Ab2 as the CL labels was established for sensitive detection of cancer antigen 125 (CA 125). As a model, sandwich CA 125-antibody was taken as molecular reorganization elements on this sensor for the sensitive determination of CA 125 in the linear range from 5.0×10^{-4} U/mL to 500 U/mL with a detection limit of 2.0×10^{-4} U/mL. The specificity, reproducibility, and stability of this sensor were also investigated. This proposed method might be attractive for clinical and biomedical applications.

1. Introduction

Graphene oxide (GO) and reduced graphene oxide (RGO) have attracted considerable interest in recent years for its remarkable properties, unique structural features, and ease of fabrication [1–4]. GO with the remarkable large specific surface area, vast oxygen-containing groups, and benign water solubility, serves as an ideal matrix for building new hybrid materials with novel characteristics [5–7]. GO hybrid materials noncovalently functionalized chemiluminescence (CL) reagents exhibites good CL activity, stability, and biocompatibility [8,9]. Meanwhile, GO is an ideal platform for immobilizing enzyme via noncovalent binding without any surface modification or coupling reagents [10]. Both CL reagent and enzyme are modified on the surface of GO sheets, the hybrid materials with unique CL features and enzymatic activity is obtained. RGO is a zero band gap material and has remarkable electroactivity and photoactivity, RGO-based hybrid structure, RGO serves as the substrate onto which 1D nanomaterials can be grown and good properties may be anticipated [11]. Graphene-mediated growth of semiconducting 1D nanomaterials heterostructures has attracted wide attention for superior electronic and optoelectronic properties. Graphene acts as a remarkable candidate to integrate with semiconducting zinc oxide nanorods (ZNRs) that may reveal distinctive properties. It is anticipated that integration of the highly conductive graphene and extreme photosensitivity of the ZNRs can enhance the optoelectronic properties of graphene-ZNRs based hybrid structures as compared to the ZNRs only.

However, the wide band gap of ZnO only allows it to absorb ultraviolet light, which limits the utilization of solar light since ultraviolet light comprises less than 5% of solar light [12]. To extend the activity of ZnO into the visible light region, the absorption range can increase up to 428 nm after CdS is anchored ZNRs [13]. The results show that CdS will play a role in generating electrons in the visible region. The CdS-ZNRs/RGO can act as an exciting active material in the field of optoelectronic device applications.

Photoelectrochemical sensor, which is just the reverse process of ECL sensor, has attracted an extensive attention in recent years [14–16]. Coupling photo-irradiation with electrochemical detection, photoelectrochemical (PEC) sensors, a newly developed and promising analytical method, have the advantages of both optical methods and electrochemical methods [17–19]. Benefiting from the total separation of excitation source and detection signal, its sensitivity could potentially match that of the ECL due to the greatly reduced background signal. In addition, the instrument should be

* Corresponding author.
E-mail address: ujn.yujh@gmail.com (J. Yu).

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simpler and lower cost than all the optical detection methods due to the use of electronic detection, particularly in an array format. Thus, this technique shows promising analytical potentials for portable, rapid, and high-throughput biological assay [20,21]. Due to its attractive potential, more efforts have been devoted to its exploitation, and substantial advances have been achieved. However, the study on PEC sensor is still in its initial phase and yet leaves much to be desired. At present, a great of works focus on exploiting photoactive materials [22,23]. In all the conventional PEC methods, to excite the photoactive materials, an external light source is required. The expensive and sophisticated light source makes the instrument complicated and departs from the portable and low-cost trend for microfluidic paper-based analytical device (μ-PADs). We exploited a PEC sensor by using an ABEI labeled CL probe as the exciting light, a new PEC strategy was constructed without an external light source.

CA-125 is an epithelial antigen that has been used as a marker for the detection of ovarian cancer. Several assays have been developed to detect CA-125 [24–26], but most are not ideal either due to lack of sensitivity or the complexity of the detection procedure. Herein, we reported a novel strategy in which we performed photoelectrochemical sensing of tumor marker CA 125. Experimentally, the Ab1 was primarily anchored onto the CdS/ZNRs/RGO-PWE for the following immunorecognition between antibody and antigen, subsequently by the labeling of ABEI-GO@HRP via Ab2 (ABEI-GO@HRP/Ab2 bioconjugates) for the final introduction of chemiluminescence reagents ABEI as internal light source into the PEC detection system, on the basis of HRP accelerated oxidation of ABEI by H_2O_2 to yield the chemiluminescence. Because of the synergy effect of the chemiluminescence as internal light source and the CdS/ZNRs/RGO hybrids as sensing platform, this present amplified PEC immunoassay has inherent higher sensitivity for the detection of CA 125 as a model protein.

2. Experimental section

2.1. Materials and instruments

The antigen and monoclonal-antibody of cancer antigen 125 (CA 125), cancer antigen-153 (CA-153), cancer antigen 199 (CA 199), neuron-specific enolase (NSE), α-fetoprotein (AFP), human chorionic gonadotropin (HCG), and carcinoembryonic antigen (CEA) were purchased from China Shanghai Linc-Bio Science Co. Ltd. ABEI was obtained from Sigma-Aldrich (U.S.A.). Zinc acetate, hexamethylenetetramine, cadmium nitrate, HRP, thiourea, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride(EDC), *N*-hydroxysuccinimide (NHS), thioglycolic acid (TGA) and *p*-iodophenol (PIP) was purchased from Aladdin Industrial Inc. (Shanghai, China). Whatman chromatography paper #1 (200.0 mm × 200.0 mm) (pure cellulose paper) was obtained from GE Healthcare Worldwide (Pudong Shanghai, China) and used with further adjustment of size. Milli-Q water obtained from a Millipore water purification system (≥18 MΩ cm, Milli-Q, Millipore) was used in all assays and solutions. Blocking buffer for blocking the residual reactive sites on the antibody immobilized paper was pH 7.4 phosphate buffer solution (PBS) containing 0.5% BSA and 0.5% casein. To minimize unspecific adsorption, 0.05% Tween-20 was spiked into 10.0 mM pH 7.4 PBS as washing buffer. All other reagents were of analytical grade and used as received.

PEC measurements were carried out with a homemade PEC system. A 500 W xenon arc lamp (CHF-XQ-500 W, Beijing Changtuo Co., Ltd.) equipped with a monochromator was used as the irradiation source. Electrochemical impedance spectrum (EIS) were carried out on an IM6x electrochemical station (Zahner, Germany). Electrochemical measurements were performed with a CHI 760 (Shanghai CH Instruments, China) system. A three-electrode system consisted of a screen-printed Ag/AgCl reference electrode and a carbon counter electrode on the paper auxiliary zone and a screen-printed carbon working electrodes (5 mm in diameter) on the paper sample zones.

2.2. Synthesis of ABEI-GO@HRP/Ab2

GO sheets were synthesized by the Hummer's method with slight modification [27,28]. The preparation of ABEI-GO@HRP/Ab2 hybrids was according to the reported reference with minor modification [8]. The preparation process of ABEI-GO@HRP/Ab2 hybrids was as follows. Firstly, 1.0 mL of 5 mM ABEI alkaline solution and 50 mL of 0.1 mg/mL GO solution was mixed to form a homogeneous solution at room temperature until the color exhibited stably dark brown, which indicated that ABEI-GO hybrids (ABEI-GO) were successfully prepared. The resulting solution was centrifuged and washed triple at a speed of 13 000 rpm for 10 min to eliminate residual ABEI and redispersed in 10 mM PBS (pH 5.6). Then 1 mg/mL HRP and 50 μg/mL Ab2 were added into the ABEI-GO hybrids solution above by the volume ratio at 1:1:10 of HRP, Ab2 and ABEI-GO, incubated at 4 °C for 9 h. Then the resulting solution was centrifuged twice to remove nonspecifically adsorbed enzyme and Ab2 was redispersed in Milli-Q water to obtain ABEI-GO@HRP/Ab2 hybrids solution.

2.3. Fabrication of ZNRs/RGO-PWE

The preparation of μ-PADs was similar to our prior work [29–31]. The fabrication of the ZNRs/RGO-PWE was as follows. First, RGO was prepared via in situ reduction of GO on the surface of cellulose fibers to prepare RGO modified PWE. 20 μL of GO dispersion (1.0 mg/mL) was added onto the paper sample zone. Second, GO-coated paper was transferred into a 50 mL Teflon lined stainless-steel autoclave. Third, the aqueous GO dispersion (1.0 mg/mL, 5.0 mL), hydrazine monohydrate (80 wt%, 20 μL), and ammonia solution (28%, 18 μL) were added into the autoclave. Subsequently, the autoclave was heated at 90 °C for 2 h, and the resultant RGO-PWE was washed triple with Milli-Q water.

ZNRs grew on RGO-PWE according to our previous work [29]. First, the seed solution was prepared by dissolving zinc acetate dehydrate (5 mM) in ethanol solution. Then, the as-prepared seed solution was dropped on RGO-PWE, dried at 120 °C to eliminate excess moisture and other solvents, which was repeated six times. An aqueous solutions composed of 25 mM zinc acetate and 25 mM hexamethylenetetramine were used as a precursor source for the growth of ZnO nanorods. The solution was adequately stirred, and then transferred into a Teflon lined stainless-steel autoclave in which ZnO seed coated RGO-PWE were placed at 90 °C for 6 h. Then, it was cooled naturally, the as-prepared ZnO/RGO-PWE was washed with Milli-Q water and ethanol respectively to eliminate unreacted materials. The substrate was allowed to dry at ambient temperature to retain the initial properties of paper.

2.4. Construction of the Ab1/CdS/ZNRs/RGO-PWE

The deposition of CdS on the ZNRs/RGO-PWE was according to the successive ionic layer adsorption and reaction method [32,33]. The electrolyte for electrodeposition was composed of 0.2 M cadmium nitrate, 0.2 M thiourea, 25 mL methanol, and 25 mL Milli-Q water. The electrodeposition was carried out with the current density of 0.5 mA cm^{-2} at 60 °C for 10 min. After the working electrode was washed with Milli-Q water and absolute ethonal, TGA (20 μL) was dropped onto the CdS/ZNRs/RGO-PWE, and reacted for 1 h. The surface ligand (thiourea) of CdS was replaced by TGA through the ligand exchange reaction. Finally, the resulting CdS/ZNRs/Au@Pt-