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Label-free electrochemiluminescent immunosensor for detection of prostate specific antigen based on mesoporous graphite-like carbon nitride

Hongmin Ma, Yanhua Zhao, Li Li, Huan Wang, Qin Wei*

Key Laboratory of Interface Reaction & Sensing Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, China

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ABSTRACT

In this paper, mesoporous graphite-like carbon nitride (mpg-C_3N_4) combined with gold nanoparticles (Au NPs) was conducted to construct a luminol-hydrogen peroxide (H_2O_2) based electrochemiluminescent (ECL) immunosensor for prostate specific antigen (PSA) detection. The mpg-C_3N_4 exhibited large specific surface area and high porosity was synthesized from a simple precursor, which possessed abundant active sites to load Au NPs and luminol (mpg-C_3N_4/Au-luminol). After that, Au NPs linked mpg-C_3N_4 (mpg-C_3N_4/Au) could hatch with the primary antibody (Ab_1) via Au-NH_2 bond, which reinforced the sensitivity of immunosensor. The mpg-C_3N_4 and Au shows excellent catalytic enhancement effect on the ECL intensity of luminol. Under optimal conditions, the constructed ECL immunosensor exhibited sensitive response to PSA in a wide linear range of 0.001 ~ 15 ng mL^{-1} with a lower detection limit of 0.927 pg mL^{-1}. Meanwhile, the proposed ECL immunosensor endows the good reproducibility and stability. Therefore, the results could open another avenue for detection of PSA and other biomarkers.

1. Introduction

Biomarkers have been utilized as important diagnostic tools for cancers and other diseases [1]. Recently, a lot of methods have been focused on the detection of cancer markers, such as fluorescence (FL) [2–5], surface plasmon resonance [6–8], chemiluminescence (CL) [9,10], mass spectrometry [11]. Prostate cancer as one of the most common malignant diseases occurs in the male prostate tissue, which is caused by abnormal growth of the prostate gland cells. Prostate specific antigen (PSA) as tumor markers is commonly used to detect prostate and breast cancer whose concentration range in normal human body is 0 ~ 4 ng mL^{-1} [12]. PSA has important clinical significance for early diagnosis, clinical staging, postoperative curative effect observation and follow-up of prostate cancer [13].

Electrochemiluminescent (ECL) as a simple, sensitive, and powerful analytical technique combines the advantages of electrochemical and luminescent techniques [14,15], involving a light emission process in a redox reaction of electrogenerated reactants. In the field of ECL, semiconductor quantum dots (QDs), $Ru(bpy)_3^{2+}$, luminol and their analogues have been usually used as luminophores [16,17]. Unlike some other luminophores, luminol/H_2O_2 is widespread studied in virtue of its high luminescence yield, chemical stability and low potential. To the best of our knowledge, most of the reported system of luminol is bound up with ROS, including superoxide radicals ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$) and HO_2^-. In order to promote the ECL efficiency of luminol/H_2O_2 system, a great deal of efforts have been devoted to produce highly reactive $O_2^{\bullet -}$ and $OH^{\bullet}$ through catalyzing H_2O_2 [18]. Thus, biosensors based on luminol systems have been widely applied to the analysis filed [19]. Ju and his co-worker [20] had constructed a quenched immunosensor which based nitrogen doped graphene catalytic reduction of oxygen to produce hydroxide, thereby blocking the ECL process of CdS dots. Taking into account the advantages of luminol, ECL immunosensor based luminol has been constructed for PSA detection in our work.

To date, graphitic-like carbon nitride (g-C_3N_4) has given rise to a great deal of attention owing to its special structure and properties, and it is widely applied in catalysis [21–25], photoelectronic devices [26–28] and chemical sensors [29–34]. As we all know, g-C_3N_4 possessed good visible-light-driven photocatalytic performance and could produce hydrogen or oxygen by water splitting under visible light, which was firstly reported [27]. Compared with g-C_3N_4, mesoporous graphitic-like carbon nitride (mpg-C_3N_4) displays much higher surface area with abundant mesoporous. mpg-C_3N_4 can expose more active sites to enhance its application in catalytic reaction performance [35]. In order to improve the photoactivity of mpg-C_3N_4, researchers have developed several methods, such as doping with metal or nonmetallic

* Corresponding author.
E-mail address: sdjndxwq@163.com (Q. Wei).

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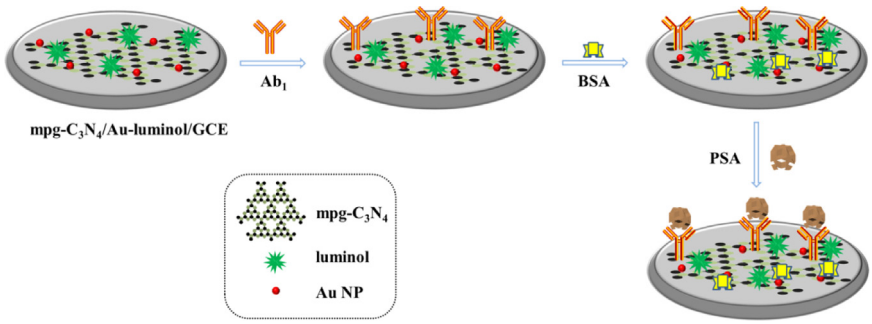

Scheme 1. The fabrication process of the ECL modified electrodes.

elements [36], and coupling with inorganic semiconductors [37,38]. Although the ECL property of g-C_3N_4 was better than mpg-C_3N_4, mpg-C_3N_4 possessed good adsorption efficiency. Herein, mpg-C_3N_4 can be set as base material to load the luminescent materials in ECL sensor construction.

In this work, mpg-C_3N_4 was synthesized via a facile one-step method to construct a luminol-H_2O_2 based ECL immunosensor for PSA detection. The fabrication process of the developed ECL immunosensor for PSA is illustrated in Scheme 1. Due to the larger specific surface area and adsorption efficiency, mpg-C_3N_4 could combine with more Au NPs and luminol. Au NPs could accelerate the electron transport to realize the sensitive detection of PSA in human serum. Furthermore, mpg-C_3N_4 as a favorable substrate material could enhance the stability of the ECL signal remarkably. Notably, mpg-C_3N_4/Au could interact with luminol/H_2O_2 system to accelerate the ECL reaction for significantly enhancing the signal of the luminol/H_2O_2 system.

2. Experimental section

2.1. Synthesis of mpg-C_3N_4

The mpg-C_3N_4 was prepared according to previous reported method [35] through pyrolysis of urea and dicyandiamide in air atmosphere. Briefly, 3.5 g urea and 1.5 g dicyandiamide was mixed and thoroughly ground in a mortar (at least 50 min until the material was in ultrafine powder). Following, the abraded powder was transferred into a crucible and then put it in a Muffle Furnace. The certain parameter of calcination was setting as follows: from room temperature to 530 °C, and the heating rate was 5 °C min^{-1}. Then, maintain for 4 h; Final, declined to room temperature with −1 °C min^{-1} and the yellow mpg-C_3N_4 powders was obtained. The g-C_3N_4 was prepared by pyrolysis of melamine under the same method.

2.2. Synthesis of Au NPs, mpg-C_3N_4/Au and mpg-C_3N_4/Au-luminol hybrids

The synthesis of Au NPs was conducted according previous methods [39]. Subsequently, 1.0 mL of mpg-C_3N_4 solution and 1.0 mL of Au NPs solution were mixed together sufficiently and then oscillation 24 h at 4 °C. After that the product was obtained by centrifugation. Finally, the product was dispersed in ultrapure water. 1.0 mL luminol was added into the above mpg-C_3N_4/Au solution and continued vibration 24 h at 4 °C. Afterward, the solution was also centrifuged and washed with ultrapure water. Finally, mpg-C_3N_4/Au-luminol hybrid were obtained and kept at 4 °C for future use.

2.3. Preparation of ECL modified electrodes

Firstly, the GCE (4 mm) was respectively polished using alumina slurry into mirror and then wished with ultrapure water. The process of modifying the GCE was shown in Scheme 1. Next, 6 μL of mpg-C_3N_4/Au-luminol hybrid was dropped and dried in room temperature. Subsequently, 6 μL of anti-PSA solution (10 μg mL^{-1}) immobilized and incubated via Au-NH_2 bond, followed by washing to remove physically absorbed Anti-PSA. Then 3 μL 1% BSA solution was incubated with the GCE to block the nonspecific binding sites and washed thoroughly. After that step, different concentrations of PSA were added to the electrode and reacted with the Anti-PSA. After rinsing thoroughly, the ECL immunosensor was fabricated successfully.

2.4. Measurement procedure

To measure the UV–vis spectra, Au NPs were obtained from the reduction $AuCl_4^-$. And g-C_3N_4/Au hybrid was prepared by a compound centrifugation of 1 mL of Au NPs solution and 1 mL of mpg-C_3N_4 solution. The concentration of mpg-C_3N_4/Au-luminol composed by 1 mL of mpg-C_3N_4/Au solution and 1 mL of 5 mmol L^{-1} luminol solution.

The immunosensors incubated with different concentrations of PSA were put in 10 mL of pH 10.0 CBS (1/15 mol L^{-1}, Carbonate buffer solution contained Na_2CO_3 and $NaHCO_3$) and 30 mmol L^{-1} H_2O_2 by single-step cycle pulse method. Pulse potential, pulse time, initial potential and pulse period were set at 0.55 V, 0.1 s, − 0.45 V and 6 s, respectively. And the scanning rate was 0.1 V s^{-1}. The ECL signal was recorded via the MPI-F flow-injection chemiluminescence detector, and the voltage of the photomultiplier tube (PMT) was set at 500 V.

3. Result and discussion

3.1. Characterization of materials

The surface compositions of the obtained mpg-C_3N_4 were characterized by XPS. The XPS survey scan spectrum (Fig. 1C) showed that the main elements of mpg-C_3N_4 were carbon and nitrogen. As shown in Fig. 1A, the C 1s spectra could be divided into three peaks at 284.5 ev, 286.1 ev and 288 ev. It could be seen that a major C peak at 288 eV, which was identified as sp^2-bonded C (C–N). The lower peak at 284.5 eV was attributed to C-C which was ordinarily observed on the XPS characterization for carbon nitrides [35]. The N 1 s spectra (Fig. 1B) could be divided into 3 peaks at 398.4 ev, 400.7 ev and 404.3 ev. The higher N1s peak at 398.4 ev was attributed to N bonded to C (sp^2 hybridized aromatic). The lower peak at 400.7 eV was owing to quaternary N—C_3 in the aromatic cycles. While the additional weak peak at 404.3 eV was arisen from the amino functional groups with hydrogen (C–N–H), which might be related to incomplete condensation [40]. Furthermore, the crystalline structure of the prepared materials was measured by XRD in Fig. 1D. The strong XRD (curve a) peak at 27.6° (002) was consistent with the reported mpg-C_3N_4 [35]. After combined with Au NPs (curve a), three new diffraction peaks appears at 38.2°, 44.4°, and 64.5°, which corresponds to the (111), (200) and (220) plane of Au.

The morphological structure of the prepared materials was investigated by the measurement of SEM. As shown in Fig. 2A, the SEM image of mpg-C_3N_4 reveals that mpg-C_3N_4 was porous structure.