Biosensors and Bioelectronics 99 (2018) 92–98

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Graphene oxide@gold nanorods-based multiple-assisted electrochemiluminescence signal amplification strategy for sensitive detection of prostate specific antigen

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ARTICLE INFO

Keywords:
GO@AuNRs-GOD
Multiple signal amplification
Streptavidin-biotin
Electrochemiluminescence aptasensor
Prostate specific antigen

ABSTRACT

A novel and competitive electrochemiluminescence (ECL) aptasensor for prostate specific antigen (PSA) assay was constructed using gold nanorods functionalized graphene oxide (GO@AuNRs) multilabeled with glucose oxidase (GOD) and streptavidin (SA) toward luminol-based ECL system. A strong initial ECL signal was achieved by electrodeposited gold (DpAu) on the electrode because of gold nanoparticles (AuNPs) motivating the luminol ECL signal. The signal probes prepared by loading GOD and SA-biotin-DNA on GO@AuNRs were used for achieving multiple signal amplification. In the absence of PSA, the signal probes can be attached on the electrode by hybridization reaction between PSA aptamer and biotin-DNA. In this state, the GOD loaded on the probe could catalyze glucose to *in situ* produce H_2O_2 and then AuNRs catalyze H_2O_2 to generate abundant reactive oxygen species (ROSs) in luminol ECL reaction. Both the high-content GOD and AuNRs in the signal probe amplified the ECL signal in the ECL system. Moreover, the combination of SA with biotin-DNA further expands ECL intensity. The integration of such amplifying effects in this protocol endows the aptasensor with high sensitivity and good selectivity for PSA detection. This aptasensor exhibits a linear relation in the range of 0.5 pg mL^{-1} to 5.0 ng mL^{-1} with the detection limit of 0.17 pg mL^{-1} (S/N = 3). Besides, the strategy was successfully applied in determination of human serum samples with recovery of 81.4–116.0%.

1. Introduction

Sensitive and accurate detection of disease–related biomarkers increasingly attracts the attention of researchers, which is critical to many areas of biomedical study and diagnosis (Kitano et al., 2002). Prostate specific antigen (PSA), a valuable serum biomarker, has been used for the early diagnosis and screening of prostate cancer (Healy et al., 2007). The rapid and quantitative detection of PSA is of great value. Currently, various measuring method including fluorescent labeling (Xu et al., 2017), electrochemical immunosensor (Kavosi et al., 2015; Li et al., 2017), organic electrochemical transistor immunosensor (Kim et al., 2010), surface plasmon resonance (Jiang et al., 2014), electrochemiluminescence aptasensor (Yang et al., 2017) have been implemented to obtain sensitive detection of PSA.

Among these methods, ECL techniques have witnessed a remarkably progress in the biosensing field owing to advantages of high detection sensitivity, easy operation, reproducibility and controllability (Zhang et al., 2017b). In the classic luminescence system, luminol-based ECL reaction has been obtained extremely attention (Cao et al., 2012). It is well known that glucose oxidase (GOD) as a biomacromolecule could efficiently catalyze glucose to *in situ* generate H_2O_2 which serves as an effective coreactant in luminol-based ECL system (Chen et al., 2010b). GOD evoked *in situ* generation strategy could well address the instability problem of the H_2O_2 directly used in the detection solution. However, owing to its weak conductivity, the electron transfer between GOD and an electrode is hard to achieve. To solve this problem, some nanocomposite films, such as reduced graphene oxide and silver nanoparticles composite film (Palanisamy et al., 2014), biomediated gold nanoparticles (AuNPs) deposited on MWCNTs-PVP composite film (Zhang et al., 2011), and MoS_2 nanosheets decorated with AuNPs composite film (Su et al., 2014) have been used. Of these nanocomposites, AuNPs play important role to achieve the purpose of both immobilizing GOD to facilitate the electron transfer and keeping the activity of GOD. In addition, it has been

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http://dx.doi.org/10.1016/j.bios.2017.07.050
Received 10 May 2017; Received in revised form 4 July 2017; Accepted 19 July 2017
Available online 20 July 2017
0956-5663/

reported that AuNPs could promote the luminescent efficiency of luminol-H_2O_2 system and thus enhance the sensitivity. The reason is that AuNPs could catalyze H_2O_2 to generate ROSs, such as hydroxyl radical ($OH^{\bullet}$) and superoxide radical ($O_2^{\bullet -}$), which are important intermediates to participate in the luminol ECL reaction.

Gold nanorods (AuNRs) have general properties similar to AuNPs and possess some advantages over the spherical AuNPs, such as superior biocompatibility, good stability, catalytic activity, etc. (Parab et al., 2010). Currently, AuNRs have been used for signal amplification via increasing the load capacity of biomolecules. For example, (Wu et al., 2015) designed an amperometric biosensor by self-assembly multilayers of AuNRs-GOD on the sol-SWCNTs matrix to detect glucose. It can be deduced that AuNRs-GOD could greatly amplify the ECL signal of luminol-H_2O_2 system. The GOD loaded on the AuNRs could catalyze glucose to *in situ* generate H_2O_2 which acts as efficient coreactant of luminol ECL system and the AuNRs thereby could hasten the decomposition of H_2O_2 to generate ROSs, producing an enhanced ECL signal. But so far the application of AuNRs-GOD on ECL aptasensor was few.

Streptavidin (SA) with strong affinity and high specificity for biotin has been widely applied in immunoassay (Heidari et al., 2014). As far as we know, each SA can combine with four biotin molecules and obviously amplify signal due to the strong interaction of SA-biotin (Lin et al., 2008). Therefore, to further improve the sensitivity, we suppose that SA and GOD could be simultaneously anchored on the AuNRs to play synergistic effect for the signal amplification.

Another approach to amplify the ECL signal of luminol-H_2O_2 is to find an efficient supporter which can effectively load AuNRs. Graphene oxide (GO) sheet is an extensively applied nanomaterial in biosensors (Rafiee et al., 2009), owing to its superior properties such as large surface area, high water solubility and electroconductivity. The GO sheets could be used as an ideal matrix for constructing various hybrid materials with new features, but poor electrical conductivity limited its practical application (Chen et al., 2012). According to the literature, decorated GO with various metal nanoparticles are widely applied in biosensors as sensing platform, greatly improving its catalytic activity (Liu et al., 2011). In addition, gold nanomaterials can also greatly increase the available surface area and biocompatibility in biosensors (Pruneanu et al., 2012). Therefore, decorated AuNRs on GO sheets may be a good choice to immobilize biomolecules and construct sensing platform.

Herein, we demonstrated a novel competitive ECL aptasensor to monitor PSA using the GO@AuNRs-GOD-SA as the signal-amplifying probes. As shown in Scheme 1, the electrodeposited gold (DpAu) with good conductivity was electrodeposited on the GCE to enhance ECL intensity. The HS-DNA (S1) was firstly hybridized with partial complementary sequence of PSA aptamer and then fixed on the electrode. Subsequently, the residual complementary sequence of PSA aptamer was binding with biotin-DNA by hybridization reaction. Upon the sandwich-type conjugation (S1-PSA aptamer-biotin-DNA), the signal probes were attached on the modified electrode, obviously amplifying luminol ECL signal by *in situ* generating coreactant H_2O_2. Due to the excellent electrical activity and the efficient catalytic effect of AuNRs, the ECL intensity enhanced. Moreover, the combination of SA with biotin-DNA can further enlarge ECL intensity. In the presence of PSA, the signal probes would fall off the electrode surface, weakening ECL response. In view of the multiple signal amplification strategy, the sensitive detection of PSA was realized.

2. Experimental

2.1. Materials and reagents

$HAuCl_4{\cdot}3H_2O$ was purchased from Sinopharm Chemical Reagent Co., Ltd. Ascorbic acid (AA, ≥ 99.7%) were obtained from Alfa Aesar (Ward Hill, MA). Luminol was obtained from Sigma-Aldrich (≥ 99.7%, St. Louis, MO, USA). Hexadecyltrimethylammonium bromide (CTAB) and glucose were purchased from Tianjin Bodi Chemical Co. Ltd. Bovine serum albumin (BSA), Human IgG (hIgG) and human serum albumins (HSA) were provided by Shanghai Solarbio Bioscience & Technology Co., Ltd. PSA and streptavidin (SA) were purchased from Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). Glucose oxidase (GOD), the 26-mer PSA binding aptamer and its two complementary strands DNA: HS-DNA (S1) and biotin-DNA (S2) were purchased from Sangon biotech Co. Ltd. (Shanghai, China). The sequences used are as follows: PSA aptamer: 5′-*T TAA TTA AAG CTC* -3′. HS-DNA (S1): 5′-*GAG CTT TAA TTA A* TTTTT-$(CH_2)_6$-SH-3′.

Biotin-DNA (S2): 5′-biotin-$(CH_2)_6$-TTTTT *G CTA TTT GAT GGC*-3′.

(The italicized part is the complementary strand of the PSA binding aptamer).

All reagents were of analytical reagent grade. All aqueous solutions were prepared using ultrapure water (Kangning Water Treatment Solution Provider, Chengdu, China).

2.2. Apparatus

ECL signals were obtained by a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) equipped with a CR 120 type photomultiplier tube (Binsong Photonics, Beijing, China). The voltage of the photomultiplier tube (PMT) was set at − 800 V. A conventional three electrode system was used for the experiment, which contained the bare or modified glassy carbon electrode (GCE, φ = 3 mm) as working electrode, an Ag/AgCl electrode as reference electrode, and a Pt wire as the counter electrode. Electrochemical measurements were monitored with a RST5200F electrochemical workstation (Zhengzhou Shiruisi Technology Co., Ltd., Zhengzhou, China). Transmission electron microscope (TEM) images were obtained with a Tecnai G^2 F20 TEM (FEI Co., Hillsboro, Oregan, USA). UV–vis absorption spectra were performed by utilizing an UV mini-1240 UV–vis spectrophotometer (Shimadzu Corp., Kyoto, Japan).

2.3. Synthesis of Au NRs

AuNRs were prepared via the seed–mediated surfactant–directed approach, and the surface of the AuNRs was wrapped with a thin layer of cetyltrimethylammonium bromide (CTAB), leading to the AuNRs being positively charged (Chen et al., 2010a). Briefly, the seed solution was synthesized by adding 0.25 mL 0.01 mol L^{-1} $HAuCl_4{\cdot}3H_2O$ into CTAB (9.75 mL, 0.1 mol L^{-1}) solution and mixed well. Then, the fresh, ice–cold $NaBH_4$ (0.6 mL, 0.01 mol L^{-1}) was quickly injected into the above solution under vigorous stirring for 2 min. Subsequently, the seed solution was kept in a 27 °C water bath for 2 h without disturbance.

To grow Au nanorods, 0.6 mL 0.01 mol L^{-1} $AgNO_3$ and 0.8 mL 1 mol L^{-1} HCl were successively added to the growth solution containing 2 mL 0.01 mol L^{-1} $HAuCl_4{\cdot}3H_2O$ and 40 mL 0.1 mol L^{-1} CTAB. Then, 0.32 mL 0.1 mol L^{-1} AA was quickly placed into and mixed for 2 min, the color became colorless immediately. Finally, 0.3 mL 2 h aged Au seed solution was injected and stirred vigorously for 2 min, and allowed to stand at 27 °C overnight. The as-synthesized AuNRs were centrifuged at 12,000 rpm for 20 min to remove excess CTAB surfactant. After discarding the supernatant, the precipitate was redispersed in 10 mL water.

2.4. Preparation of GO@AuNRs and GO@AuNRs-GOD-SA-biotin-DNA nanocomposites

Graphene oxide (GO) was synthesized from natural graphite powder using a modified Hummers' method (Hummers and Offeman, 1958). The GO@AuNRs nanocomposite was prepared based