Analytica Chimica Acta 853 (2015) 234–241

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

A sensitive electrochemical aptasensor based on palladium nanoparticles decorated graphene–molybdenum disulfide flower-like nanocomposites and enzymatic signal amplification

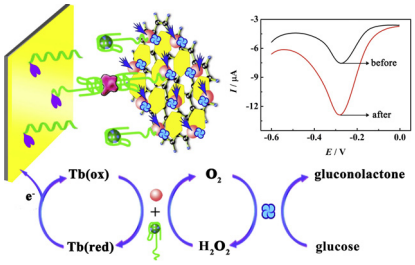
Pei Jing, Huayu Yi, Shuyan Xue, Yaqin Chai, Ruo Yuan *, Wenju Xu *

Key Laboratory of Luminescent and Real-Time Analytical Chemistry, Ministry of Education (Southwest University), School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

HIGHLIGHTS

- PDDA–G–MoS_2 nanoflowers were firstly used for the fabrication of thrombin aptasensor.
- MoS_2 was adopted to enhance the surface area of graphene and accelerate the electron transfer.
- GOD, PdNPs and hemin/G-quadruplex could amplify the electrochemical signal through synergetic catalysis.
- The proposed aptasensor displayed an improved sensitivity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 July 2014
Received in revised form 26 September 2014
Accepted 6 October 2014
Available online 13 October 2014

Keywords:
Aptasensor
Graphene–molybdenum disulfide
Electrochemical response
Signal amplification
Thrombin

ABSTRACT

In the present study, with the aggregated advantages of graphene and molybdenum disulfide (MoS_2), we prepared poly(diallyldimethylammonium chloride)–graphene/molybdenum disulfide (PDDA–G–MoS_2) nanocomposites with flower-like structure, large surface area and excellent conductivity. Furthermore, an advanced sandwich-type electrochemical assay for sensitive detection of thrombin (TB) was fabricated using palladium nanoparticles decorated PDDA–G–MoS_2 (PdNPs/PDDA–G–MoS_2) as nanocarriers, which were functionalized by hemin/G-quadruplex, glucose oxidase (GOD), and toluidine blue (Tb) as redox probes. The signal amplification strategy was achieved as follows: Firstly, the immobilized GOD could effectively catalyze the oxidation of glucose to gluconolactone, coupling with the reduction of the dissolved oxygen to H_2O_2. Then, both PdNPs and hemin/G-quadruplex acting as hydrogen peroxide (HRP)-mimicking enzyme could further catalyze the reduction of H_2O_2, resulting in significant electrochemical signal amplification. So the proposed aptasensor showed high sensitivity with a wide dynamic linear range of 0.0001 to 40 nM and a relatively low detection limit of 0.062 pM for TB determination. The strategy showed huge potential of application in protein detection and disease diagnosis.

1. Introduction

As artificially selected oligonucleotides (DNA or RNA) [1,2], aptamers can specifically bind to numerous target proteins with high affinity, and have been widely employed in sensors for ultrasensitive detection by using various analytical protocols [3–8].

* Corresponding authors. Tel.: +86 23 68252277; fax: +86 23 68253172.
E-mail address: xwju@swu.edu.cn (W. Xu).

http://dx.doi.org/10.1016/j.aca.2014.10.003
0003-2670/

Among these methods, electrochemical aptasensors have attracted particular attention because of the merits of fast response, high sensitivity, simple operation, miniaturization and relatively low cost [9–11]. In addition, for the purpose of further improving the sensitivity of the electrochemical aptasensors, enzyme labeling amplification strategy has been extensively adopted to realize the ultrasensitive detection of targets [12,13], owing to the fact that enzyme performs as transducer to convert biomolecular recognition event into electrochemical signal and intrinsic catalytic activity to achieve the signal amplification.

Besides, graphene, as a two-dimensional (2D) nanomaterial with predominant physical and chemical properties, has attracted great attentions for bioassays [14,15]. Recently, molybdenum disulfide (MoS_2) with the ability to availably induce more complicated planar electric transportation properties [16], a typical family number of transition metal dichalcogenides, possesses similar layered structure of graphene and has become a research hotspot [17–20]. Furthermore, the combination of MoS_2 and other conducting carbon nanomaterials has attracted extensively attention [21,22], because the combined nanocomposites exhibit synergistic effects, such as perfect conductivity, larger surface area and excellent electrochemical performance. The synergistic effects endow these nanocomposites ability to maintain the intriguing activity of the redox probes and enzymes, which provides a general perspective in the construction of novel biosensors.

Thrombin (TB), an extracellular serine protein, plays significant roles in the blood coagulation cascade, haemostasis and thrombosis [23,24]. So, it is extremely crucial to realize ultrasensitive and specific detection of TB. Herein, a sandwich-type electrochemical aptasensor for TB was developed based on palladium nanoparticles decorated poly (diallyldimethylammonium chloride)–graphene/molybdenum disulfide flower-like nanocomposites(PdNPs/PDDA–G–MoS_2) and enzyme catalysis reactions. As the aggregation of graphene and MoS_2, PDDA–G–MoS_2 was synthesized by a facile hydrothermal method [25] and firstly acted as platform to immobilize biomolecules. Through the electrostatic attractive force, the negatively charged palladium nanoparticles (PdNPs) were decorated on the surface of the positively charged PDDA–G–MoS_2. With large specific surface areas and more active sites, the prepared PdNPs/PDDA–G–MoS_2 served as nanocarriers for highly dense conjugation of redox-active toluidine blue (Tb), glucose oxidase (GOD), and hemin/G-quadruplex as HRP-mimicking DNAzyme [26] with the final formation of hemin/G-quadruplex conjugated Tb-PdNPs/PDDA–G–MoS_2 (secondary aptamer), owing to the strong interaction between the amine groups (NH_2) of biomolecules with PdNPs [27]. The target protein TB was sandwiched between the primary aptamer and the prepared secondary aptamer. After adding glucose, the immobilized GOD effectively catalyzed the oxidation of glucose to gluconolactone with the production of H_2O_2, which was further electrocatalyzed by PdNPs and hemin/G-quadruplex. Thus, the electron transport of Tb was promoted, resulting in significant enhancement of the electrochemical signal. Experiment results showed that this signal amplified strategy could effectively realize the quantitative detection of TB with a broad linear range and a relative low detection limit, which offered a promising way for simple, rapid and sensitive detection of other proteins in research and clinical applications.

2. Experimental

2.1. Reagents and material

Graphene oxide (GO) was obtained from Nanjing XianFeng Nano Co. (Nanjing, China). $Na_2MoO_4{\cdot}2H_2O$ and poly(diallyldimethylammonium chloride) (PDDA) were purchased from Beijing Chemical Reagent Co. (Beijing, China). Carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) was obtained from Biocell Company (Zhengzhou, China). Thrombin (TB), glucose oxidase (GOD), toluidine blue (Tb), hemin, palladium potassium chloride (K_2PdCl_4), gold chloride ($HAuCl_4$), hemoglobin (Hb), bovine serum albumin (BSA), L-cysteine (L-cys) and human IgG were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Glucose was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Thrombin aptamer (TBA): 5′-NH_2-$(CH_2)_6$-GGTTGGTGTGGTTGG-3′ was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Trishydroxymethylaminomethane hydrochloride (Tris–HCl) was provided by Roche (Switzerland). The human serum samples were obtained from the Xinqiao Hospital (Chongqing, China).

Tris–HCl buffer (20 mM, pH 7.4) was prepared with 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM KCl and 140 mM NaCl, used as aptamer buffer. Phosphate buffered solution (PBS) with different pH was served as working buffer throughout the experiment, containing 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4 and 0.1 M KCl. The prepared solutions were kept at 4 °C before use. All of the other chemicals were of analytical grade and directly used as received. Double distilled water was used throughout this experiment.

2.2. Apparatus

The electrochemical measurements were carried out with a CHI-660D electrochemical workstation (Shanghai Chenhua Instrument, China) with a three electrode system comprised of a platinum wire auxiliary, a saturated calomel electrode (SCE) reference and the modified glass carbon (GCE, Φ = 4 mm) working electrode. The size and morphology of materials were taken with a scanning electron microscope (SEM, S-4800, Hitachi, Tokyo, Japan). X-ray photoelectron spectroscopy (XPS) analysis was performed using the Thermo ESCALAB 250Xi spectrometer with Al Ka X-ray (1486.6 eV) as the light source (Thermoelectricity Instruments, USA). pH measurement was performed by a pH meter (MP 230, Mettler-Toledo, Switzerland).

2.3. Synthesis of PdNPs decorated PDDA–G–MoS_2 flower-like nanocomposites (PdNPs/PDDA–G–MoS_2)

Initially, PDDA functionalized graphene–MoS_2 flower-like nanocomposites (PDDA–G–MoS_2) were prepared via a hydrothermal method according to the previous protocol [25]. 0.0086 g GO was ultrasonically dispersed in 10 mL double distilled water. Then, 0.075 g $Na_2MoO_4{\cdot}2H_2O$ and 75 μL PDDA (20 wt%) were added into GO solution. After ultrasonication and stirring for 20 min, 0.2 g thiourea was added into the mixture and diluted with double distilled water to 20 mL under vigorously stirring for 1 h. Subsequently, the obtained solution was maintained at 200 °C for 24 h in a 25 mL teflon-lined stainless-steel autoclave. After cooling at room temperature, the black product was gathered via centrifugation, washed with ethanol and double distilled water alternately for several times, and dried at 60 °C in a vacuum drying oven.

PdNPs were attached to PDDA–G–MoS_2 by electrostatic adsorption between negatively charged PdNPs and positively charged PDDA–G–MoS_2. 5 mg PDDA–G–MoS_2 was dispersed in 5 mL double distilled water by sonication. Then, 1 mL K_2PdCl_4 (10 mM) was wisely dropped into the dispersion and vigorously stirred for 10 min. In sequence, 1 mL freshly prepared $NaBH_4$ (0.1 M) was slowly added into the mixture and the resulting mixture stirred for 30 min. Following that, the prepared PdNPs/PDDA–G–MoS_2 was collected by centrifugation and washed for several times with double distilled water.

For the comparison of different loading capability, PDDA-reduced graphene oxide (PDDA-rGO) nanocomposites were synthesized according to reference [28] by using hydrazine