Biosensors and Bioelectronics 94 (2017) 616–625

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Ultrasensitive electrochemical biosensing platform based on spherical silicon dioxide/molybdenum selenide nanohybrids and triggered Hybridization Chain Reaction

Hong-Lei Shuai[a,c], Xu Wu[b,c], Ke-Jing Huang[a,c,*], Zi-Bo Zhai[a,c]

[a] College of Chemistry and Chemical Engineering, Xinyang Normal University, Xinyang 464000, China
[b] School of Physics and Electronic Engineering, Xinyang Normal University, Xinyang 464000, China
[c] Institute for Conservation and Utilization of Agro-bioresources in Dabie Mountains, Xinyang Normal University, Xinyang 464000, China

ARTICLE INFO

Keywords:
Spherical silicon dioxide/molybdenum selenide
Hybridization Chain Reaction
Sandwich-type
Electrochemical biosensor
Signal amplification
Graphene oxide–gold nanoparticles

ABSTRACT

An ultrasensitive sandwich-type electrochemical biosensor for DNA detection is developed based on spherical silicon dioxide/molybdenum selenide (SiO_2@$MoSe_2$) and graphene oxide–gold nanoparticles (GO–AuNPs) hybrids as carrier triggered Hybridization Chain Reaction (HCR) coupling with multi-signal amplification. The proposed sensoring assay utilizes a spherical SiO_2@$MoSe_2$/AuNPs as sensing platform and GO–AuNPs hybrids as carriers to supply vast binding sites. H_2O_2+HQ system is used for DNA detection and HCR as the signal and selectivity enhancer. The sensor is designed in sandwich type to increase the specificity. As a result, the present biosensor exhibits a good dynamic range from 0.1 fM to 100 pM with a low detection limit of 0.068 fM (S/N=3). This work shows a considerable potential for quantitative detection of DNA in early clinical diagnostics.

1. Introduction

Recently, ultrasensitive and high-selective detection of low abundance nucleic acids has attracted considerable interest due to its important role in precaution of genetic or pathogenic diseases, treatment of viral and bacterial infections, and prognosis of cancer (Liu et al., 2016; Zhou et al., 2014). Various analytical technologies, such as chemiluminescence (Wang et al., 2015a, 2011; Li et al., 2017; He et al., 2013), spectrophotometry (Patel et al., 2016), fluorescence (Ye et al., 2016; Wang et al., 2014a; Wei et al., 2016; Zhu et al., 2015), surface enhanced Raman scattering (Duan et al., 2015; Li et al., 2013) and electrochemical methods (Li et al., 2016; Wang et al., 2015b, 2014b; Yuan et al., 2016) have been used for DNA detection. Among these methods, electrochemical DNA sensors have received particular attention owing to the superior features of low cost, simple, portable and rapid response (Wan et al., 2013; Yao et al., 2013; Yu et al., 2016). Many signal amplification strategies have been used in electrochemical biosensor construction for improving the sensitivity of target DNA detection (Yu et al., 2016). Hybridization Chain Reaction (HCR) is one of the most important (Shuai et al., 2016a; Li et al., 2015; Guo et al., 2016; Trifonov et al., 2016). In the process of HCR, target acts as an initiator to trigger the hybridization reaction, issuing in the formation of long-range DNA sequence in a long nicked duplex DNA. The initiator triggered reaction and generates a low pseudo-positive result, leading to a high signal to noise ratio. In addition, every target can trigger a HCR event and form a long-range DNA sequence. These advantages exhibit great potential in the sensitive detection of DNA.

Nanomaterials with many specific properties have been widely applied in the field of DNA detection. Two-dimensional (2D) transition metal dichalcogenides, such as MoS_2, WS_2, CoS_2 and VS_2, can be good choices due to their large specific surface areas, good electrical conductivity and chemical stability (Huang et al., 2016, 2014). As a typical 2D layered material, MoS_2 can provide short ion diffusion length and large exposed surface area, and therefore has received considerable attention for electrochemical applications (Huang et al., 2014; Farimani et al., 2014). However, the electrical conductivity of MoS_2 is not that good, and $MoSe_2$ with the similar layered structure might be a better candidate. $MoSe_2$ has a higher intrinsic electrical conductivity than MoS_2 due to the more metallic nature of Se, and the unsaturated Se-edges in $MoSe_2$ are also found to be electrocatalytically active (Kong et al., 2013; Wang et al., 2013). However, because of the high surface energy and interlayer Van der Waals attraction, restacking of $MoSe_2$ nanosheets is almost unescapable in practical applications, which greatly reduces the effective specific surface area, and therefore weakens its performance in some degree (Chhowalla et al., 2013; Hwang et al., 2011). So, assembling primordial 2D $MoSe_2$ nanosheets

* Corresponding author at: College of Chemistry and Chemical Engineering, Xinyang Normal University, Xinyang 464000, China.
E-mail address: kejinghuang@163.com (K.-J. Huang).

http://dx.doi.org/10.1016/j.bios.2017.03.058
Received 14 January 2017; Received in revised form 22 March 2017; Accepted 27 March 2017

onto a template is very significative. In the past few years, SiO_2 nanospheres have been researched extensively for its unique properties including high surface area, inertness, low cost, good hydrophility, simple preparation process, easy surface functionalization, and good biocompatibility (Chen et al., 2015; Ensafi et al., 2016).

Graphene oxide (GO) has high surface area, good water dispersibility and biocompatibility and has been widely used in the construction of electrochemical biosensors (Tung et al., 2016; Shuai et al., 2016b; Borisova et al., 2016). AuNPs are always utilized as conductive enhancer due to its good conductivity. So, GO–AuNPs hybrid will be very suitable to be a signal amplification carrier. This hybrid will greatly improve the probe capacity and heighten the electron mobility, thus enhance the detection sensitivity of the fabricated biosensor.

Herein, an ultrasensitive sandwich-type electrochemical biosensor for DNA detection is developed based on spherical SiO_2@$MoSe_2$ and GO–AuNPs hybrids. Triggered HCR, enzyme and the catalytic reaction of hydrogenperoxide+hydroquinone system are used for signal amplification. Spherical SiO_2@$MoSe_2$ with high surface area and good conductivity are utilized as sensing substrate, which can load more AuNPs to immobilize capture probe and accelerate the electron transfer rate. GO–AuNPs hybrids act as signal amplification carrier can supply abundant Au-S binding sites to conjugate probe DNA. When the target DNA shows up, HCR will occur between auxiliary DNA and bio-H1–bio-H2 (H1–H2), resulting in signal amplification. In addition, horseradish peroxidase (HRP) enzymes are also immobilized on the electrode as a signal indicator via specific binding of avidin-biotin. Signal-amplification can be further achieved through the catalytic reaction of hydrogenperoxide+hydroquinone (H_2O_2+HQ) system. Therefore, combining the above advanced aspects together, the proposed assay shows ultrahigh sensitivity and selectivity for target DNA detection.

2. Experimental section

2.1. Reagents and materials

Graphite powder, selenium powder, concentrated ammonia solution, hydrazine hydrate, tetraethoxysilane (TEOS), $Na_2MO_4{\cdot}2H_2O$, urea and chloroauric acid ($HAuCl_4{\cdot}3H_2O$) were obtained from Aladdin Chemicals Co. Ltd. (Shanghai, China). 6-mercaptohexanol (MCH) was purchased from Sigma-Aldrich (Shanghai, China). Water was purified with a Milli-Q purification system (≥18 MΩ, Millipore). Horseradish peroxidase (HRP) and all DNA sequences were synthesized by Shanghai Sangon Biological Engineering Technology Co. Ltd. (Shanghai, China) and their sequences were as follows:

Capture probe: 5’ -ACGGAAACTGCAAGTATTTTT-(CH2)3-SH -3’
Probe DNA: 5’-SH - (CH2)6-TGCAGTTTCCGTCCGTAGTTTTT -3’
Target DNA: 5’-CTACGGACGGAAACTGCACCTGTATTCCCATACCCATCAT-3’
One-based mismatch DNA: 5’-CTACGGACGGAAACTGCAACTGTATTCCCATACCCATCAT-3’
Three-based mismatch DNA: 5’-CTACGGACGCAAACTGCAACTGTATTCTCATACCCATCAT-3’
Noncomplementary: 5’-CTGCTTCCAAACCTTTAACATAGCCGCAAGCGTTAGCTGC-3’
Auxiliary DNA: 5’-AGTCTAGGATTCGGCGTGGGTTAAATGATGGGTATGGGAATACAGG-3’
Bio-H1: 5’-biotin-TTAACCCACGCCGAATCCTAGACTCAAAGTAGTCTAGGATTCGGCGTG-3’
Bio-H2: 5’-biotin-AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCT

The buffers used in this work are shown in Table 1.

Table 1
Buffers used in this work.

No.	Buffer	Application
1	PBS1 (10 mM PBS, 3 M NaCl and 2.7 mM KCl, pH 7.4)	Capture DNA, Probe DNA
2	PBS2 (10 mM PBS, 150 mM NaCl, 2.7 Mm KCl and 10 mM MgCl2, pH 7.4)	Target DNA, Auxiliary DNA, Mismatch DNA
3	PBS3 (50 mM PBS and 1 M NaCl, pH 7.4)	Bio-H1, Bio-H2

2.2. Apparatus

Cyclic Voltammetry (CV) and differential pulse voltammetry (DPV) were tested on an EC550 electrochemical workstation (Wuhan, Gaoss Union, China) and electrochemical impedance spectroscopy (EIS) measurements were carried out on a RST5200F electrochemical workstation (Zhengzhou Shi Rui Si Instrument, China) with a conventional three-electrode system composed of a platinum wire as an auxiliary electrode, a saturated calomel electrode (SCE) as a reference electrode and a 3-mm diameter GCE as a working electrode. Nanostructures were characterized by a JEM 2100 transmission electron microscope (TEM, JEOL, Tokyo, Japan) and a Hitachi S-4800 scanning electron microscope (SEM, Tokyo, Japan). The BET specific surface areas of the samples were analyzed by using a nitrogen adsorption apparatus (ASAP2460 4MP, China). X-ray diffraction (XRD) pattern was operated on a model D/max-rA diffractometer (Rigaku, Japan). Raman spectra were recorded at ambient temperature on a Renishaw Raman system model 1000 spectrometer (Gloucestershire, UK).

2.3. Preparation of GO–AuNPs hybrids

Graphene oxide (GO) was prepared through modified Hummers’ method. GO–AuNPs carrier was prepared according to a previous protocol (Goncalves et al., 2009). Firstly, 25 mL of 0.48 mM $HAuCl_4$ solution and 1.25 mL of 1 mg/mL GO solution were mixed and stirred for 30 min at room temperature. After that, the mixture was heated to 80 °C, and 470 μL of 85 mM sodium citrate was then added into the solution slowly and stirred for 60 min. Finally, the sample was obtained after the reaction mixture was centrifugally (5 min, 8000 rpm) washed with water and dried in drying oven at 60 °C.

Typical immobilization of DNA on GO–AuNPs hybrids were as follow (Wang et al., 2016): firstly, probe DNA (20 μL, 100 μM) were mixed with 1 mL GO–AuNPs solution and incubated for at 4 °C 48 h. Next, the mixture was centrifuged for 10 min at 8000 rpm. The precipitate was washed with 10 mM PBS (pH=7.0) for several times and then dispersed in 1 mL of 10 mM PBS (pH=7.0).

2.4. Synthesis of spherical SiO_2@$MoSe_2$

In order to obtain spherical $MoSe_2$, SiO_2 was firstly prepared as template. 24.8 mL water and 90 mL concentrated ammonia solution were mixed to obtain solution A. 61.8 mL absolute ethyl alcohol and 4.5 mL TEOS were mixed uniformly to obtain solution B. Then, solution B was dropwise added into solution A under vigorous stirring for 2 h. The white precipitate of SiO_2 was centrifugally washed with water and ethyl alcohol, and finally dried in drying oven at 60 °C.

To grow hierarchical $MoSe_2$ shell on SiO_2 sphere template, 0.05*g* of the as-prepared SiO_2 sphere was ultrasonically dispersed into a mixture of 20 mL water and 30 mL ethyl alcohol. Then, 0.21g sodium molybdate ($Na_2MoO_4{\cdot}2H_2O$) was added and ultrasonic for 10 min. After that, 0.18g selenium powder which was dissolved in 10 mL hydrazine hydrate was added to the reaction solution. At last, the mixture was transferred into a 100 mL Teflon-lined stainless steel autoclave and sustained in an oven at 200 °C for 24 h. The black precipitate of SiO_2@