Biosensors and Bioelectronics 70 (2015) 122–129

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Label-free impedimetric aptasensor for detection of femtomole level acetamiprid using gold nanoparticles decorated multiwalled carbon nanotube-reduced graphene oxide nanoribbon composites

Airong Fei [1], Qian Liu [1], Juan Huan, Jing Qian, Xiaoya Dong, Baijing Qiu, Hanping Mao, Kun Wang *

Key Laboratory of Modern Agriculture Equipment and Technology, School of Chemistry and Chemical Engineering, Jiangsu University, Zhenjiang 212013, PR China

ARTICLE INFO

Article history:
Received 23 January 2015
Received in revised form
11 March 2015
Accepted 12 March 2015
Available online 13 March 2015

Keywords:
Impedimetric aptasensor
Gold nanoparticle
Graphene oxide nanoribbon
Acetamiprid

ABSTRACT

Gold nanoparticles (Au NPs) decorated multiwalled carbon nanotube-reduced graphene oxide nanoribbon (Au/MWCNT-rGONR) composites were synthesized by a one-pot reaction. By employing the resulting Au/MWCNT-rGONR composites as the support for aptamer immobilization, we developed an ultrasensitive label-free electrochemical impedimetric aptasensor for acetamiprid detection, which was based on that the variation of electron transfer resistance was relevant to the formation of acetamiprid–aptamer complex at the modified electrode surface. Compared with pure Au NPs and MWCNT-rGONR, the Au/MWCNT-rGONR composites modified electrode was the most sensitive aptasensing platform for the determination of acetamiprid. The proposed aptasensor displayed a linear response for acetamiprid in the range from 5×10^{-14} M to 1×10^{-5} M with an extremely low detection limit of 1.7×10^{-14} M (S/N=3). In addition, this impedimetric aptasensor possessed great advantages including the simple operation process, low-cost, selectivity and sensitivity, which provided a promising model for the aptamer-based detection with a direct impedimetric method.

1. Introduction

Acetamiprid, as a kind of neonicotinoids with low acute and chronic mammalian toxicity, has been widely used as the replacement insecticide of organophosphorus and other conventional insecticides to preventing numerous sucking insects in agricultural product in our country (Shi et al., 2013). However, owing to its frequent and extensive usage, recent studies have shown that the acetamiprid released in the surface or ground water and accumulated in the soil would still cause potential risk of human health (Pramanik et al., 2006; Ragas et al., 2011; Sanyal et al., 2008). Therefore, it is necessary to develop analytical methods for detection of acetamiprid in environment to keep people from potential health risk. The conventional analytical methods including high performance liquid chromatography (Xie et al., 2011), gas chromatography (Zhang et al., 2008) and enzyme-linked immunosorbent assays (Kim et al., 2004) are powerful for acetamiprid detection, however, these techniques suffer from high cost, long processing time, and requirement of technical skills and sophisticated equipments (Xu et al., 2011). As a result, looking for a more simple and sensitive method for fast and convenient acetamiprid determination is meaningful and has application prospect for practical environmental monitoring.

Aptasensors, which are based on the result of its selective binding to the aptamer immobilized on the electrode surface, have been explored as a promising method for the detection of a target molecule (Bonanni et al., 2012; Wu et al., 2014). Previous reports concerning aptasensors were usually relied on the chemical modification of aptamers for the labeling of fluorophores or nanoparticles, which were rather costly, time-consuming, and sophisticated (Huang et al., 2013). Recently, as an alternative strategy for the rapid and sensitive quantification of target analytes, label-free electrochemical impedimetric aptasensors have been proved to be one of the most powerful analytical tools for interfacial investigation due to the advantages in terms of simplicity, sensitivity and noninvasiveness (Fayazfar et al., 2014; Wang et al., 2012). By the combination of the electrochemical impedance spectroscopy (EIS) and aptasensing technique, numerous impedimetric aptasensors have been designed in recent years for determination of

* Corresponding author. Fax: +86 511 88791708.
E-mail address: wangkun@ujs.edu.cn (K. Wang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bios.2015.03.028
0956-5663/

proteins such as thrombin (Deng et al., 2009; Qi et al., 2013), cytochrome c (Ocaña et al., 2014), and phycotoxin such as ochratoxin A (Evtugyn et al., 2013) and okadaic acid (Eissa et al., 2013). For example, based on the specific interaction between the thrombin molecules and its aptamer, Deng et al. (2009) developed a simple and ultrasensitive label-free impedimetric aptasensor for selective thrombin detection; through monitoring of the okadaic acid binding induced conformational change within the aptamer, Eissa et al. (2013) achieved a label-free aptasensor with low detection limit for okadaic acid detection based on electrochemical impedance spectroscopy. All these results above indicated that the impedimetric aptasensors exhibited improved performance in the analytical applications by combination the advantages of EIS with aptasensor, however, investigations on exploring impedimetric aptasensing platform for pesticide detection in environmental has been rarely reported to date.

As we all know, the accuracy and sensitivity of the aptasensors were influenced directly by the amount of the immobilized aptamer at the surface of an electrode, therefore, it plays an important role to increase the amount of the immobilized aptamer in the fabrication of the aptasensors (Wu et al., 2014). According to previous reports, nanostructure materials with large surface area and abundant binding points have been employed as the electrode materials to construct aptasensing platforms, such as gold nanoparticles (Au NPs) (Li et al., 2013), carbon nanotubes (Zelada-Guillén et al., 2009), and graphene-based nanomaterials containing graphene oxide (Loo et al., 2012), graphene oxide/C_3N_4 nanocomposites (Li et al., 2014a), Au NPs decorated graphene sheets (Hu et al., 2011a), 3,4,9,10-perylene tetracarboxylic acid functionalized graphene sheets (Hu et al., 2011b), and etc, which were beneficial to increase the immobilization amount of aptamer greatly and further obtain the amplified electrochemical detection signals. With the purpose of enhancing the sensitivity of aptasensor for acetamiprid detection, herein we developed a facile label-free impedimetric aptasensor for detection of acetamiprid at femtomole level using the Au NPs decorated multiwalled carbon nanotube-reduced graphene oxide nanoribbon (Au/MWCNT-rGONR) composites, which was more sensitive for acetamiprid detection than pure Au NPs and MWCNT-rGONR, respectively. Based on the combination of electrochemical impedimetric method with aptamer, the impedimetric aptasensor exhibited improved performance with wider linear range, lower detection limit and high selectivity for acetamiprid detection.

2. Experimental

2.1. Reagents

Acetamiprid (99%, purity) was obtained from Sigma-Aldrich (USA). MWCNT-GONR was synthesized from MWCNTs (obtained from Shenzhen Nanotech Port Co. Ltd) according to our previous report (Liu et al., 2015). Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), sodium citrate, 6-mercapto-1-hexanol (MCH), Tris hydroxymethyl aminomethan (Tris), and ethylenediaminetetraacetic acid disodium salt (EDTA) were purchased from Sinopharm Chemical Reagent Co., Ltd. Aptamer with the following sequence: 5′-(SH)-$(CH_2)_6$-TGT AAT TTG TCT GCA GCG GTT CTT GAT CGC TGA CAC CAT ATT ATG AAG A-3′ was purchased from Sangon Biotech Co., Ltd (Shanghai, China). Aptamer stock solutions were prepared with 50 mM Tris–HCl buffer (pH 7.4, containing 0.1 M NaCl, 0.2 M KCl, 5.0 mM $MgCl_2$ and 1.0 mM EDTA) and kept frozen in dark. Phosphate buffered solution (PBS, 0.1 M) of various pH values were prepared by mixing stock standard solutions of NaH_2PO_4 and Na_2HPO_4, and adjusted the pH with 0.1 M NaOH or H_3PO_4 solution. All chemicals and solvents were at least of analytical grade and used as received without further purification. All solutions were prepared with twice-distilled water.

2.2. Apparatus

Transmission electron microscopy (TEM) image was taken with a JEOL 2100 TEM (JEOL, Japan) operated at 200 kV. The UV–vis and Raman spectra of the samples were obtained from a Perkin-Elmer Lambda 18 UV–vis-NIR spectrometer (USA) and RM 2000 microscopic confocal Raman spectrometer (Renishaw, England), respectively. X-ray photoelectron spectrometry (XPS) analyses were carried out on an ESCALAB MKII X-ray photoelectron spectrometer (V.G. Scientific. Ltd, England). A conventional three-electrode system was employed with a saturated calomel electrode (SCE) as the reference electrode, a platinum wire as the counter electrode, and the modified glassy carbon electrodes (GCE, $\Phi = 3$ mm) as the working electrodes. EIS was performed in 0.1 M PBS containing 5 mM $Fe(CN)_6^{3-/4-}$ and 0.1 M KNO_3 with a frequency range from 0.1 Hz to 100,000 Hz, and the amplitude of the applied sine wave potential in each case was 5 mV which was taken with a ZENNIUM electrochemical workstation (Zahner Instruments, Germany). All the potentials in this work were with respect to SCE. All measurements were carried out at room temperature (20 ± 2 °C)

2.3. Preparation of Au/MWCNT-rGONR composites

Au/MWCNT-rGONR composites were synthesized by a one-pot reaction. In a typical synthesis, 30 mg of sodium citrate was added to a refluxed and stirred homogeneous MWCNT-GONR dispersion (25 mL, 0.1 mg/mL) and the solution was further refluxed and stirred for 2.5 h, and then 100 μL $HAuCl_4$ (1 wt.% in water) was quickly added to the above solution and reflux was continued for another 30 min. Finally, the resulting homogeneous black dispersion was centrifuged at 13000 r/min and washed with twice-distilled water for several times. Subsequently, the Au/MWCNT-rGONR composites were re-dispersed into 5 mL twice-distilled water by sonication for further use.

2.4. The aptasensor fabrication and its detection mechanism

Prior to modification, the GCE was first polished with sand paper followed by 1.0, 0.3, and 0.05 μm malumina slurry, respectively, and sequentially sonicated for 1 min in ethanol and double-distilled water bath to remove any residues, respectively. The procedure for the construction of the aptasensor was as follows: firstly, the pretreated GCE surface was modified by dropping 6 μL of 0.5 mg/mL Au/MWCNT-rGONR composites suspension and dried in air at room temperature to form Au/MWCNT-rGONR composites modified GCE (denoted as Au/MWCNT-rGONR/GCE), then the electrode was immersed in aptamer solution (10 μM) in order to assemble the aptamer on the electrode surface. The assembly electrode was stored in the refrigerator at 4 °C overnight, followed by rinsing with Tris–HCl buffer for several times to remove physically absorption. Then, the electrode was dried in a nitrogen stream, after which the interface was covered with 6 μL of 1 mM MCH and kept at room temperature for 1 h to block nonspecific sites, followed by rinsing with ethanol and twice-distilled water respectively. Finally, the MCH/aptamer/Au/MWCNT-rGONR/GCE was obtained, as displayed in Scheme 1. For comparison, the MCH/aptamer/Au NPs/GCE and MCH/aptamer/MWCNT -rGONR/GCE were prepared by the similar procedure.

The detection mechanism of the aptasensor toward acetamiprid was as follows: in the presence of target acetamiprid, the obvious increment of EIS signal was obtained due to the formation of acetamiprid–aptamer complex at the modified electrode surface, and the increase of EIS signal depended on the concentration