Talanta 134 (2015) 699–704

Contents lists available at ScienceDirect

Talanta

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

A new electrochemical aptasensor based on electrocatalytic property of graphene toward ascorbic acid oxidation

Liang Wu [a,b], Erhu Xiong [a], Yue Yao [a], Xia Zhang [a], Xiaohua Zhang [a,*], Jinhua Chen [a,*]

[a] State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, P.R. China

[b] College of Chemistry and Life Science, Zhejiang Normal University, Jinhua 321004, China

ARTICLE INFO

Article history:
Received 15 August 2014
Received in revised form
8 December 2014
Accepted 11 December 2014
Available online 18 December 2014

Keywords:
Graphene
Nanocatalyst
Ascorbic acid
Adenosine triphosphate
Electrochemical aptasensor

ABSTRACT

Based on the superior electrocatalytic property of graphene (GN) toward ascorbic acid (AA) oxidation, a new electrochemical aptasensor has been developed. Here, adenosine triphosphate (ATP) is used as the model to demonstrate the performance of the developed aptasensor. Briefly, GN is attached to the thiolated ATP binding aptamer (ABA) modified gold electrode through π-π stacking interaction, resulting in a significant oxidation signal of AA. In the presence of ATP, the formation of ATP-ABA complex leads to the release of GN from sensing interface, resulting in a sharp decrease of the oxidation peak current of AA and an obviously positive shift of the related peak potential. Taking both the change values of the peak current and peak potential of AA oxidation as the response signals, ATP can be detected sensitively. This is the first time to demonstrate the application of GN as the nanocatalyst in an amplified aptasensor. It can be expected that GN, as nanocatalyst, should become the very promising amplifying-elements in DNA-based electrochemical biosensors.

1. Introduction

Graphene (GN) is a new form of carbon nanomaterials with single-atom-thick and two-dimensional structure [1]. Over the past years, GN and its related nanomaterials have been widely used in the DNA-based electrochemical sensors due to their good biological compatibility, high surface area and excellent electrochemical properties [2,3]. They are usually used as the electrode materials with good conductivity and abundant binding points [4–6], the carriers for loading numerous signal elements [7,8], the tracers based on their direct electrochemistry [9–11], and the mediators to regulate the electron transfer process [12]. For example, Akhavan et al. utilized the vertical reduced graphene nanowalls (RGNWs) as the porous electrode materials for an ultra-high-resolution electrochemical detection of the four bases of DNA [13]. Pumera et al. used graphene oxide nanoplatelets (GONPs) as the inherently electroactive tracers for DNA analysis [14]. Dong developed the impedimetric biosensors for the detection of ATP or Hg^{2+} based on the ultrahigh electron transfer ability of graphene [15]. Actually, GN and its related nanomaterials are also the preeminent nanocatalysts toward many substances, such as H_2O_2, ascorbic acid (AA), uric acid (UA) and dopamine (DA), etc [16,17]. In John's work, the electrochemically reduced graphene oxide (ERGO) showed the excellent electrocatalytic activity [18]. It not only enhanced the oxidation currents of AA, DA, and UA, but also shifted the oxidation potentials of them to less positive potentials compared to the bare glassy carbon electrode (GCE) and separated their electrochemical oxidation signals into three distinct peaks.

As we known, electrocatalysis represents a preferred means for signal amplification in electrochemical biosensors. Thanks to their superior electrocatalytic properties, many nanomaterials, especially the noble metal nanoparticles, have been widely employed as the signal amplification elements in DNA-based electrochemical biosensors [19–22]. Willner's group developed the amperometric biosensor for the amplified electrochemical detection of DNA and protein based on the Pt nanoparticles toward electrocatalytic reduction of H_2O_2 [23]. Cai et al. reported a new electrochemical strategy for the detection of hepatitis C virus using the electrocatalytic signal amplification method of Au nanoparticles combining with a conformation-switched hairpin DNA probe [24]. In general, nanocatalysts used in electrochemical sensors have many advantages, such as their high effective surface area offers a large number of active sites and often a high signal-to-noise ratio, their catalytic properties lead to a decrease in the overpotential needed for a reaction to become kinetically viable and their good conductivity causes a high rate of electron transport [25,26]. Moreover, nanocatalysts can also overcome the problems associated with the thermal and environmental instability inherent in biological materials (such as enzymes). Unfortunately, there is no report taking GN as the nanocatalyst for signal amplification in the

* Corresponding authors. Tel.: +86 731 88821848.
E-mail addresses: mickyxie@hnu.edu.cn (X. Zhang), chenjinhua@hnu.edu.cn (J. Chen).

http://dx.doi.org/10.1016/j.talanta.2014.12.011
0039-9140/

DNA-based electrochemical biosensors, although GN possesses excellent electrocatalytic property.

Aptamers are DNA or RNA molecules selected from random-sequence nucleic acid libraries using systematic evolution of ligands by exponential enrichment (SELEX). They have specific binding affinity to a variety of target molecules, such as drugs, organic or inorganic molecules, proteins, even cells [27]. Adenosine triphosphate (ATP) is generally acknowledged as the "energy currency" in most animate beings, which plays very important role in most enzymatic activities [28]. DNA-based ATP aptamer can bind two ATP molecules in a noncanonical, but stable, helix comprised of G:G and G:A base pairs flanked by short canonical helices [29].

In this paper, ATP and its aptamer were used as the model and a new electrochemical aptasensor has been developed based on the superior electrocatalytic property of GN to AA oxidation. As shown in Scheme 1, ATP binding aptamer (ABA) was firstly immobilized onto Au electrode via the Au-S bond (ABA/Au) [30,31]. Then, the aptamer could strongly adsorb GN due to the π-π interaction between GN and ABA (GN/ABA/Au) [32,33]. Because of the excellent catalytic property of GN, the obtained GN/ABA/Au electrode has showed a significantly electrocatalytic signal of AA oxidation. Upon the target binding, the formation of ATP-ABA complex led to the release of GN from the sensing interface, resulting in the decrease of the loading amount of nanocatalyst on the electrode. Such a conformational change not only results in the decrease of the oxidation peak current of AA, but also the positive shift of the related peak potential. Thus, sensitive detection of ATP can be realized by simultaneously monitoring the changes of the peak current (ΔI) and peak potential (ΔE) of AA. To our best knowledge, this is the first time to demonstrate the application of GN as the nanocatalyst in an amplified aptasensor. It can be expected that GN should become the very promising amplifying-elements in other DNA-based electrochemical biosensors.

2. Materials and methods

2.1. Reagents and materials

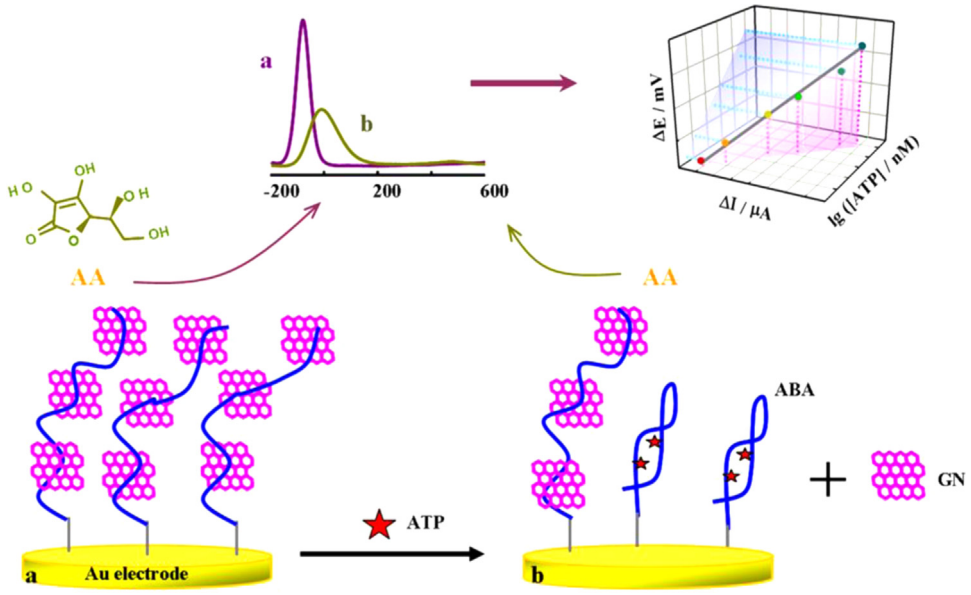
Graphene oxide (GO) was prepared from natural graphite powder through a modified Hummers method [34]. GN was prepared by chemical reduction of GO with hydrazine according to the literature [35]. Procedures were as follows: 240.0 μL of ammonia solution (25.0 wt %) and 14.5 μL of hydrazine hydrate (50.0 wt %) were added to 0.5 mg mL^{-1} GO (20.0 mL). After being vigorously stirred for a few minutes, the glass vial containing the above mixture was put in a water bath (95 °C) with continuously stirred for 1 h. Then, the product (GN) was centrifuged and washed with ultra pure water for several times, and dried in a vacuum oven at 60 °C for 48 h. Finally, a certain amount of GN was dispersed in ultra pure water with ultrasonication to prepare 0.5 mg mL^{-1} GN solution.

The ABA and another DNA strand which is not the ATP aptamer (NABA) were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequence of ABA is 5'–SH-ACCTGGGGGAGTATTGCGGAG-GAAGGT-3' according to the literatures [15,36]. The sequence of NABA is 5'–SH-AGGAGCGCGAGAAGTGTGGTGCATGTG-3'. DNA stock solutions were prepared with Tris–HCl buffer (25.0 mM Tris–HCl, 150.0 mM NaCl, pH=7.4) and kept frozen. ATP, cytidine triphosphate (CTP), guanosine triphosphate (GTP) and uridine triphosphate (UTP) were obtained from Sangon Biotechnology Co. Ltd. (Shanghai, China). AA was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). The AA solution (1.0 mM) was freshly prepared in phosphate buffer solutions (10.0 mM PBS, 100.0 mM NaCl, pH 7.4) prior to use. All other chemicals were of analytical grade and used without further purification. Aqueous solutions used throughout were prepared with ultra pure water (>18 MΩ cm) obtained from a Millipore system.

2.2. Apparatus

All electrochemical measurements were performed on a CHI 660B Electrochemical Workstation (Chenhua Instrument Company of Shanghai, China). A conventional three-electrode cell was used with a planar gold (Au) electrode (2 mm in diameter) as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode.

A definite volume of the GN solution was dropped on the mica surface and air-dried, and then the morphology of GN was characterized by atomic force microscope (AFM, MultiMode, Veeco Instruments Inc., USA) in the tapping-mode at room temperature. The prepared GN powder was further characterized by Raman spectroscopy and the related Raman spectra were recorded on a Renishaw inVia Reflex micro-Raman spectrometer with 633 nm laser excitation.



Scheme 1. Schematic illustration for a simple electrochemical ATP aptasensor based on the graphene as nanocatalyst.