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Label-free aptamer biosensor for thrombin detection based on functionalized graphene nanocomposites

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ABSTRACT

A label-free and amplified electrochemical impedimetric aptasensor based on functionalized graphene nanocomposites (rGO–AuNPs) was developed for the detection of thrombin, which played a vital role in thrombosis and hemostasis. The thiolated aptamer and dithiothreitol (TBA15–DTT) were firstly immobilized on the gold electrode to capture the thrombin molecules, and then aptamer functionalized graphene nanocomposites (rGO–TBA29) were used to fabricate a sandwich sensing platform for amplifying the impedimetric signals. As numerous negative charges of TBA29 on the electrode repelled to the $[Fe(CN)_6]^{4-/3-}$ anions, resulting in an obvious amplified charge-transfer resistance (R_{ct}) signal. The R_{ct} increase was linearly proportional to the thrombin concentration from 0.3 to 50 nM and a detection limit of 0.01 nM thrombin was achieved. In addition, graphene could also be labeled with other probes via electrostatic or π–π stacking interactions to produce signals, therefore different detection methods expanding wide application could be used in this model.

1. Introduction

Graphene has been a new star for application in various fields, such as catalysis [1], energy storage [2] and electrochemical biosensors [3] since its discovery in 2004. The applications of graphene in biosensors are focused on several fields. First, graphene or functionalized graphene has been used to modify the substrate such as GCE [4–6], ITO device [7], and quartz chip [8] because of their excellent conductivity and high chemical stability. Then graphene or graphene oxide can also act as a quencher to quench the fluorescence of illuminant for its unique electronic properties [9–11]. Li and his coworkers have realized real-time target monitoring in living cells by graphene oxide [10]. Last but not the least, graphene has high surface area; more and more graphene-based nanocomposites with different kinds of functions as enhanced sensing material have been reported [7,12,13].

Aptamers, which are singled-stranded oligonucleotides, possess high recognition ability to specific targets ranging from small inorganic, organic molecules even to proteins, cells or mycotoxins [14–16]. Since systematic evolution of ligands by an exponential enrichment (SELEX) process was firstly reported by Tuerk and Gold [17], various advanced methods for obtaining the aptamers have been developed. Aptamers exhibit multifarious advantages such as easy production, excellent controllability and versatility over the traditional recognition elements [18–22]. As a result, many aptamer-based methods have been used for the detection of proteins including quartz crystal microbalance (QCM) [23–25], surface plasmon resonance (SPR) [23,26], fluorescence [27,28], colorimetry [29], electrochemiluminescence (ECL) [30,31], electrochemistry [32,33], and so on. Among them, electrochemistry aptasensors have been widely used in medical, biological and environmental analyses. Especially, label-free electrochemical aptasensors have been developed rapidly due to their simplicity, convenience, low cost, etc. [34–36].

Here, we describe a label-free electrochemical impedimetric aptasensor for the determination of thrombin based on graphene–gold nanoparticle hybrids with enhanced sensitivity and selectivity for the aptasensors. Thrombin (TB), which plays a vital role in thrombosis and hemostasis [37], was chosen as a model protein in this work. And high sensitive detection of TB is essential for diagnosis. Because one TB molecule has two active sites for its aptamers (TBA15 and TBA29) [38], an electrode-TBA15/TB/TBA29-functionalized graphene nanocomposites sandwich system was fabricated as the sensing platform. With the aim to offer a significant amplification for the impedimetric detection of TB, reduced graphene oxide with gold nanoparticles (rGO–AuNPs) was used as a signal enhancer by covalently binding

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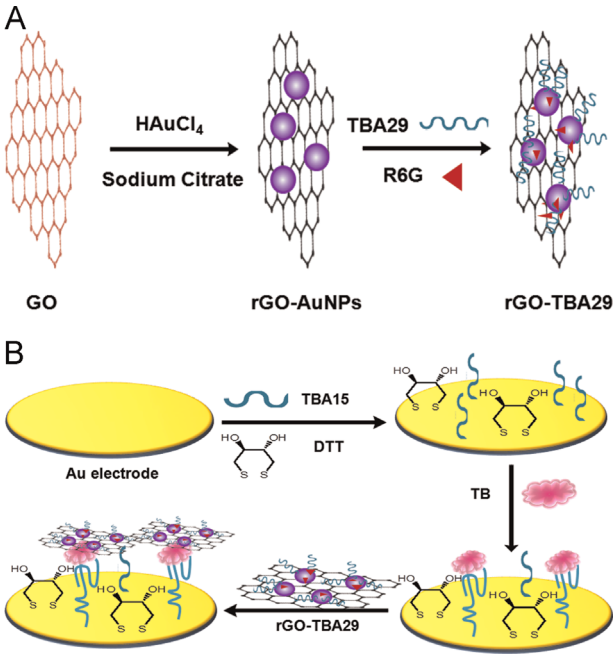

Fig. 1. (A) Illustration of the procedure for preparing rGO–AuNPs and rGO–TBA29 hybrid materials. (B) Schematic illustration of the impedimetric aptasensor with rGO–AuNPs as a signal amplified platform.

TBA29. For preparation, TBA15 and dithiothreitol (TBA15–DTT) were firstly immobilized on the gold electrode, followed by capturing the target TB, then the TBA29 functionalized reduced graphene oxide (rGO–TBA29) could further bind to TB to form a sandwich sensing system on the electrode as shown in Fig. 1. By using an electrochemical impedance spectroscopy (EIS) method, an efficient amplified charge-transfer resistance (R_{ct}) was obtained because hundreds of negatively assembled TBA29 repelled the $[Fe(CN)_6]^{4-/3-}$ anions. This approach not only presents a simple and general model for signal amplification of the impedimetric sensor but also offers a promising signal amplified model for protein detection. Because graphene could be labeled with other probes via electrostatic or π–π stacking interactions to produce signals, this model could also apply to other different methods such as SPR, ECL and so on. As a result, this aptasensor provides a very sensitive and promising detection model in the field of bioassay.

2. Experimental

2.1. Materials and chemicals

Graphene oxide (GO) was synthesized from natural graphite powder by a modified Hummers method [39]. Dithiothreitol (DTT) was obtained from Sigma-Aldrich. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) was bought from Bio Basic Inc. (Markham Ontario, Canada). Rhodamine6G (R6G) was obtained from Fluka (Buchs, Switzerland). Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) and Tris (hydroxymethyl)aminomethane (Tris) were purchased from Shanghai Chemical Reagent Company (Shanghai, China). All other chemicals were of analytical grade. Ultrapure water was used throughout the study.

Thiolated thrombin aptamers TBA15 (5′-HS-SH-$(CH_2)_6$-TTT TTT TTG GTT GGT GTG GTT GG-3′) and TBA29 (5′-HS-$(CH_2)_6$-TTT TTT TTA GTC CGT GGT AGG GCA GGT TGG GGT GAC T) were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). Thrombin, bovine serum albumin (BSA), Trypsin, immunoglobulin G (IgG) were obtained from Sigma-Aldrich. The proteins and DNA were prepared in 34 mM Tris–HCl buffer (pH 7.4, 233 mM NaCl, 8.5 mM KCl and 3.4 mM $MgCl_2$) and stored at 4 °C before use.

2.2. Apparatus

EIS measurement was performed on a Zahner Zennium electrochemical workstation (ZAHNER-Elektrik GmbH & Co.KG, Germany) and CV measurement was performed with a model CH Instrument 832B electrochemical workstation (Shanghai Chenhua Equipments, China). A conventional three electrode electrochemical cell was used here with a Ag–AgCl reference electrode, a bare gold electrode (1.2 mm in diameter) as a working electrode and a platinum wire as a counter electrode. Both EIS and CV measurements were carried out at room temperature in the solution of 5 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ in Tris–HCl buffer, and EIS was performed under an oscillation potential of 5 mV over the frequency range of 1 MHz to 0.1 Hz. High-resolution transmission electron microscopy (HRTEM) measurements were performed on a JEM-2100F high-resolution transmission electron microscope operating at 200 kV. Transmission electron microscopy (TEM) was performed on a HITACHI H-600 Analytical TEM with an accelerating voltage of 100 kV. X-ray photoelectron spectroscopy (XPS) measurement was performed on an ESCALAB-MKII spectrometer (VG Co., United Kingdom) with Al Kα X-ray radiation as the X-ray source for excitation.

2.3. Synthesis of rGO–AuNPs

The rGO–AuNPs material was synthesized in a one-pot reaction according to the reported literature [40]. Briefly, 60 mg of sodium citrate was added to homogeneous GO dispersion (50 mL, 0.1 mg mL^{-1}) under refluxing and stirring. The mixture was further refluxed and stirred for 2.5 h, whereupon 100 μL $HAuCl_4$ (2 wt% in water) was quickly added to the above solution and reflux was continued for another 30 min. Finally, the resulting homogeneous black rGO–AuNPs dispersion was centrifuged at 12,000 rpm and washed with water. Subsequently, the material was redispersed into 6 mL water by sonication for further use.

2.4. Preparation of rGO–TBA29

The TBA29 functionalized reduced graphene oxide was prepared according to the procedure of literature [41] with a little modification. Detailedly, 20 μL of TCEP (1 mM in water) and excess of TBA29 was added to 100 μL of as-prepared rGO–AuNPs suspension and incubated for 24 h before diluting to 0.5 mL with water. After centrifugation at 12,000 rpm for 10 min twice to remove the free DNA, 100 μL of 0.1 mM R6G used to block the remained space of rGO–AuNPs surface was added to the above mixture overnight. Then rinsed with water again and stored the mixture at 4 °C before use.

2.5. Fabrication of the aptasensor

Prior to aptamer immobilization, the gold electrode (1.2 mm in diameter) was polished with 1.0 and 0.3 μm alumina slurry respectively, and ultrasonically washed with water, ethanol and water. Then the electrode was electrochemically cleaned in 0.1 M H_2SO_4 by potential scanning between −0.2 and 1.6 V until a reproducible cyclic voltammetry was obtained. Finally, the electrode was rinsed thoroughly with water and dried under nitrogen gas.

For immobilization of the aptamer, 10 μL of TBA15 solution (5 μM with 200 μM DTT in Tris–HCl buffer) was placed on the cleaned gold electrode with a plastic cap overnight (about 10 h) at room temperature. Then, Au/TBA15–DTT interface was immobilized with 10 μL concentration of TB respectively, or 1 μM non-