Sensors and Actuators B 227 (2016) 655–659

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Study on rolling circle amplification of Ebola virus and fluorescence detection based on graphene oxide

Jia Wen[a], Weisi Li[a], Jiaqi Li[a], Binbin Tao[a], Yongqian Xu[a], Hongjuan Li[a], Aiping Lu[b,*], Shiguo Sun[a,*]

[a] College of Science, Northwest A&F University, Yangling, Shaanxi 712100, PR China
[b] School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, 999077 Hong Kong, PR China

ARTICLE INFO

Article history:
Received 18 August 2015
Received in revised form 4 January 2016
Accepted 10 January 2016
Available online 13 January 2016

Keywords:
Rolling circle amplification
Ebola virus
Graphene oxide
Fluorescence

ABSTRACT

A graphene oxide (GO) assisted rolling circle amplification (RCA) platform for Ebola virus (EBOV) detection is developed with simplicity and high sensitivity. In the absence of EBOV gene, no RCA products generated and the fluorescein amidate (FAM) labeled detection probe was adsorbed on the surface of GO, resulting in fluorescence quenching of the FAM. Addition of the EBOV gene allowed RCA to be taken place and the formation of double-stranded DNA (dsDNA) between RCA products and FAM labeled detection probe, leading to desorption of the FAM labeled detection probe from GO surface accompanied fluorescence recovery. EBOV gene can be determined both in aqueous solution and 1% serum solution. The limit of detection was 1.4 pM.

1. Introduction

The highly sensitive and selective detection of ultra-low concentrations of pathogenic gene plays an important role in fundamental research and practical applications such as clinical diagnosis, drug tracking, gene therapy, biomedical research, environmental analysis and food safety [1–3].

To improve the detection sensitivity, lots of sensing and signal amplification methods have been developed. Among them, polymerase chain reaction (PCR) is considered as the most successful method, which has been widely applied in the detection of DNA [4–6]. Unfortunately, PCR needs complicated thermal cycling steps and requirement of tedious and expensive equipment. Moreover, it may introduce false positive results from non-specific amplification because of the complicated thermal cycling steps. To compensate this, rolling circle amplification (RCA), an isothermal amplification technique in which a polymerase extends the length of a DNA molecule by several orders of magnitude using a circular primer as the template [7], has been developed and extensively used due to its simplicity, low-cost, high speed, high efficiency and practicability [8,9]. In recent years, a variety of RCA amplification and detection methods based on fluorescence detection have been reported for nucleic-acid diagnostics [7,10–23], the detection of RNA [24–27], protein [28] and so on. Unfortunately, most of the fluorescent probes exhibit their own inevitable background signal which may interfere with the detection result and also the sensitivity. In order to overcome this, dye-modified molecular beacon (MB) has been developed for fluorescence sensing systems based on RCA [10,27,29–32]. While, the dye-modified MB would bring about complexity and high cost which may limit its application to some extent. It is still urgent to find a more simple and convenient fluorescent RCA detection method, fluorescence sensing systems based on graphene oxide (GO) can be a good candidate.

GO, a single-atom-thick 2D nanomaterial, has been widely used for biological applications such as DNA analysis, protein assay, virus detection, drug delivery, live cell imaging, owing to its extraordinary electronic, optical, and thermal properties [33–36]. It has been demonstrated that GO can interact with single-stranded DNA (ssDNA) by π–π stacking interaction between the nucleotide bases and GO, but hardly interacts with rigid double-stranded DNA (dsDNA) or aptamer–target complexes [29,34,37–39]. Most notably, GO has been reported to be an efficient quencher for fluorescent molecules and has been employed as fluorescence quencher based on the energy transfer mechanism or the photoinduced electron transfer mechanism [40,41]. As demonstrated in our previous work, the fluorescence of dye can be efficiently quenched by GO or its derivatives, and then it can be successfully restored with the addition of a certain amount of dsDNA [39,42–44], this has been employed to detect ssDNA and dsDNA based on the lin-

* Corresponding authors.
E-mail addresses: aipinglu@hkbu.edu.hk (A. Lu), sunsg@nwsuaf.edu.cn (S. Sun).

http://dx.doi.org/10.1016/j.snb.2016.01.036
0925-4005/

ear relationship existed between the fluorescence recovery and the dsDNA concentration.

In continuation of our research, we developed a facile and simple method for the detection of Ebola virus (EBOV), a severe virus caused fatal illness in human [45,46], based on the combination of RCA and the fluorescence quenching property of GO.

As shown in Scheme 1, in the absence of EBOV gene, RCA cannot take place and no RCA products were generated. Fluorescein amidate (FAM) labeled detection probe was adsorbed on the surface of GO, resulting in the fluorescence quenching of the FAM. Upon the addition of EBOV gene, RCA carried on and dsDNA between the RCA products and the FAM labeled detection probe are formed which leads to desorption of the FAM labeled detection probe from GO surface and fluorescence recovery. In this way, EBOV gene can be determined both in aqueous solution and 1% serum solution.

2. Experimental

2.1. Materials

Natural flake graphite (325 mesh) was purchased from Alfa Aesar Co., Ultrapure Milli-Q water ($p > 18.0\,M\Omega\,cm$) was used throughout the fluorescence experiments. Exonucleases I and III were obtained from Takara Biotechnology (Dalian) Co., Ltd. (China). T_4 DNA ligase, dNTPs and phi 29 DNA polymerase were purchased from New England Biolabs (England). The human serum sample was supplied by Hospital of Northwest A&F University. And all of the experiments were performed in compliance with the relevant laws and institutional guidelines, and were approved by Northwest A&F University. DNA oligonucleotides with different sequences were synthesized and HPLC purified by Beijing DingGuo Biotech., Co., Ltd. (Beijing, China). The sequences of the oligonucleotides used in this study are as follows:

- Padlock probe: 5′-phosphate CTGCTGGTAGTACACCTATAACGACGAGAA GGGCTGCCAGATACTCTTCGCAATTTTCCGTCTGGCG-3′
- Primer: 5′-TGGCAGCCCTTTCTC-3′
- EBOV gene: 5′-CTACCAGCAGCGCCAGACGG-3′
- Detection probe: 5′-FAM-GGGCTGCCAGATACTCTTCGCAATTTT-3′
- Mismatch sequence 1 (one-base mismatched target): 5′-CTACCAGCAGCCCCAGACGG-3′
- Mismatch sequence 2 (three-base mismatched target): 5′-CTACCAGCAGGCGCAGACGG-3′
- Mismatch sequence 3 (five-base mismatched target): 5′-CTACCAGCTCGCGCAGACGG-3′

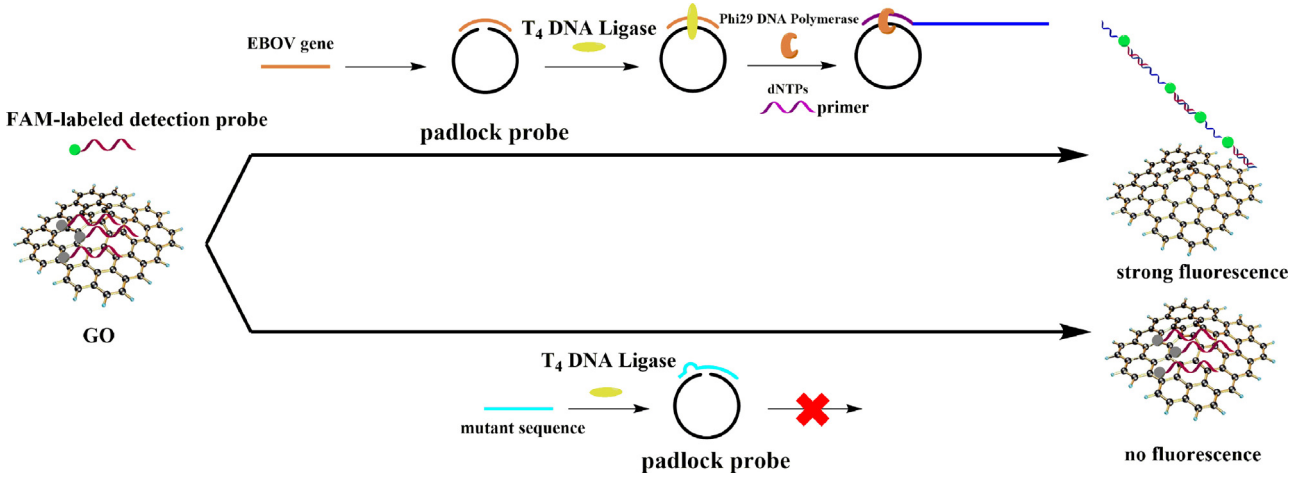
Other chemicals are all of analytical grade and were used without further purification.

2.2. Preparation of GO

GO was fabricated according to the literature we have published [39,44]. The graphite powder (2 g) was put into cold (0 °C) concentrated H_2SO_4 (46 mL). $KMnO_4$ (6 g) was added gradually with stirring and cooling, so that the temperature of the mixture was not allowed to reach 20 °C. The mixture was then stirred at 35 °C for 2 h, and distilled water (90 mL) was added. In 15 min, the reaction was terminated by the addition of a large amount of distilled water (280 mL) and 30% H_2O_2 solution (20 mL), after which the color of the mixture changed to bright yellow. The mixture was filtered and washed with 1:10 HCl solution in order to remove metal ions. Then the product was dried for 48 h at 50 °C in air, obtaining GO. The X-ray diffraction (XRD) pattern of GO is presented in Fig. S4. The as-prepared GO has a layered structure with a basal spacing of 0.82 nm, showing the complete oxidation of graphite into the graphite oxide, confirming the successful fabrication of GO [47].

2.3. Amplified detection of EBOV gene

Firstly, 1 μL of 30 μM padlock probe DNA and different concentrations of the EBOV gene were added in 32.6 μL ultrapure water with 3.4 μL ligation buffer solution (50 mM Tris–HCl, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, pH 7.5). The concentration of oligonucleotides was determined spectrophotometrically. The mixture was incubated at 95 °C for 10 min, then 55 °C for 2 h and cooled down to room temperature. Next, 400 U of T_4 DNA ligase as well as 9 μL ultrapure water and 50 μL ligation buffer solution were added and the mixture was incubated at 16 °C for 2 h to complete the ligation of padlock probe. After ligation, T_4 DNA ligase was inactivated by heating the reaction mixture at 65 °C for 20 min. To digest the leftover ssDNA and dsDNA to acquire closed DNA, 40 U of exonuclease I and 40 U of exonuclease III were added to the mixture and incubated at 37 °C for 30 min. Then the enzymes were denatured by heating at 65 °C for 5 min. The prepared circular DNA could be used directly or stored at −20 °C. Secondly, 4 μL of the circular DNA fabricated from different concentrations of EBOV gene, 20 U of the phi 29 DNA polymerase, 0.8 μL 100 × BSA and 2 μL of 10 mM dNTPs and 10 μL of primer were mixed to a final volume of 40 μL in polymerase buffer (50 mM Tris–HCl, 10 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 4 mM DTT, pH 7.5). The mixture was incubated at 30 °C overnight. The resulting RCA products could be used directly or stored at −20 °C.



Scheme 1. Schematic illustration of the GO assisted amplified biosensor for EBOV detection.