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Lable-free quadruple signal amplification strategy for sensitive electrochemical p53 gene biosensing

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ABSTRACT

A versatile label-free quadruple signal amplification biosensing platform for p53 gene (target DNA) detection was proposed. The chitosan–graphene (CS–GR) modified electrode with excellent electron transfer ability could provide a large specific surface for high levels of AuNPs-DNA attachment. The large amount of AuNPs could immobilize more capture probes and enhance the electrochemical signal with the excellent electrocatalytic activity. Furthermore, with the assist of N.BstNB I (the nicking endonuclease), target DNA could be reused and more G-quadruplex-hemin DNAzyme could be formed, allowing significant signal amplification in the presence of H_2O_2. Such strategy can enhance the oxidation–reduction reaction of adsorbed methylene blue (MB) and efficiently improve the sensitivity of the proposed biosensor. The morphologies of materials and the stepwise biosensor were characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and cyclic voltammetry (CV). Differential pulse voltammetry (DPV) signals of MB provided quantitative measures of the concentrations of target DNA, with a linear calibration range of 1.0×10^{-15}–1.0×10^{-9} M and a detection limit of 3.0×10^{-16} M. Moreover, the resulting biosensor also exhibited good specificity, acceptable reproducibility and stability, indicating that the present strategy was promising for broad potential application in clinic assay.

1. Introduction

The p53 gene, a tumor suppressor, plays critical roles in many cellular anti-cancer mechanisms and is known as “the guardian of genome” (Lee et al., 2009; Chen et al., 2010). If this gene is mutated, it can speed up tumor growth. The development of a feasible, reliable and sensitive strategy for the detection of p53 gene, especially at ultra-low physiological concentrations, has been of great significance. Owing to its inherent advantages such as simplicity, sensitivity, rapidness and low cost, to date, considerable attentions have been given to the electrochemical technique in DNA detection (Paleček and Bartošík, 2012). Furthermore, in order to realize the ultrasensitive detection, many efforts have been made for developing novel electrochemical biosensors based on various target amplification and signal amplification principles.

As for the target amplification, the polymerase chain reaction (PCR) method for DNA monitoring exhibits limitations such as complexity, potential contamination and high cost (Lee et al., 1993). The rolling circle amplification (RCA) shows some improvement, but still encounters problems such as hash reaction conditions and costly polymerase (Li et al., 2010). On this occasion, nuclease assisting target recycling was put forward. So far, various smart nuclease including restriction endonucleases (Chen et al., 2013; Zhu et al., 2014) and exonucleases (Wang et al., 2014; Liu et al., 2013a, 2013b) have been applied for bioanalytical applications. Among them, the nicking endonuclease signal amplification (NESA) as a newly developed technique attracted researchers' attention to design sensitive biosensors of target DNA for their autonomous circular amplification and simple operations (Miao et al., 2013; Zhang et al., 2014b). As one of the nicking endonuclease, N.BstNB I enzyme cleaves only one strand of a double stranded DNA four bases away from the 3'-end of its recognition sequence (5′-GAGTC-3′), which have been used extensively (Zhou et al., 2013).

On the other hand, signal amplification principles often realized by nanomaterials. One major merit is that one can control and tailor their properties in a predictable manner to meet the

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needs of specific applications. Furthermore, the integration of nanomaterials with functional DNA has provided new hybrid systems (Rosi and Mirkin, 2005). Among various nanomaterials, gold nanoparticles (AuNPs), with huge reactive surface area, unusual catalytic property and biocompatibility, were often used to prepare aptamer-conjugated AuNPs for detection of biomolecules (Wu et al., 2013; Deng et al., 2013; Jungemann et al., 2013). And the formed DNA-AuNP conjugates have been utilized as amplifying labels for novel biosensing protocols owning to their unique physical and chemical features, such as multivalent binding capability, enhanced affinity, excellent catalytic properties for signal amplification (Qiu et al., 2013; Xu et al., 2013).

In order to further push down the detection limit, another nanomaterial, graphene (GR), with the structure of a single-atom-thick sheet of sp^2-bonded carbon atoms in a closely packed honeycomb two-dimensional lattice, has draw a lot of attention. Considering its biocompatibility (Chen et al., 2008), GR is an ideal material utilized for improved performance of biosensors attributed to synergistic effect of GR and accompanying material in the nanocomposite (Yao et al., 2012). Furthermore, the high surface area of GR is helpful in increasing the surface loading of the molecules. Because of the low electronic noise from thermal effect, GR-based chemical sensors can also have a much higher sensitivity (He et al., 2012). Moreover, the excellent conductivity and small band gap are favorable for conducting electrons from the biomolecules (Brownson et al., 2012). However, GR tends to form agglomerates. Thus, the successful dispersion of GR has enabled the construction of various potentially useful GR-based biosensors. Chitosan (CS), a semi-synthetically derived aminopolysaccharide with many admirable properties (Erdem et al., 2014; Singh et al., 2013), is commonly used to disperse nanomaterials for constructing biosensors (Kang et al., 2009). As it is a polycationic polymer (Erdem et al., 2014), it can adhere to negatively charged GR to form a well dispersed and stable CS–GR nanocomposite. Furthermore, the existence of CS on GR can provide a good biocompatible microenvironment (Kang et al., 2009).

More recently, considerable research efforts have been directed toward the application of DNAzymes, a new effective biocatalyst in biorecognition and biosensing events as an electrocatalytic label (Pelossof et al., 2010, 2012; Zhou et al., 2012). As one of the most classic DNAzyme, G-quadruplex-hemin complex, which is composed of a single-stranded guanine-rich nucleic acid and hemin, has horseradish peroxidase-like activity and shows great catalytic activity to H_2O_2 (Deng et al., 2008). Several advantages over natural peroxidases, such as their small size, high chemical and thermal stability, low cost and easy synthesis (Breaker, 2000) makes G-quadruplex-hemin complex a very attractive biocatalysts for amplified biosensing applications and a novel kind of catalytic label for the development of DNA biosensors (Liu et al., 2014; Gao and Li, 2013). Moreover, many researchers have combined methylene blue (Zhang et al., 2014a), alcohol (Zheng et al., 2014), thionine (Yuan et al., 2015) with G-quadruplex-hemin to construct novel biosensing platform, in which the changes of current are triggered by the catalysis of G-quadruplex-hemin to H_2O_2 and NADH.

To significantly improve the detection sensitivity for p53 gene, herein, a highly sensitive and selective sensing strategy based on the combination of quadruple signal amplifications, AuNPs, GR, the N.BstNB I-assisted target recycling and G-quadruplex-hemin DNAzyme catalyzing was proposed. By the assisting of the excellent signal generation catalyzed by the G-quadruplex-hemin DNAzyme, our method is exempt from conventional cumbersome labeling and is essentially simple and convenient by exploiting the inherent advantages of electrochemistry as well. The proposed label-free and quadruple signal amplification approach for DNA detection showed a high sensitivity, wide detection range and excellent specificity, and thus possesses promise for sensing application.

2. Experimental section

2.1. Materials and reagents

Methylene blue (MB) was purchased from Aladdin. Tris-(2-carboxyethyl) phosphine (TCEP), tris(hydroxymethyl) aminomethane (Tris) and hemin were obtained from Sangon Biotech Co., Ltd. Chitosan (CS), acetic acid and glutaraldehyde (GLU) were purchased from Tianjin reagent chemicals Co., Ltd. Graphite powder was provided by Qingdao Fujin graphite Co., Ltd. Graphene (GR) was synthesized by the modified Hummers method (Wang et al., 2012). Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. AuNPs (about 13 nm) were prepared by means of citrate reduction of $HAuCl_4$ according to the literature (Liu and Lu, 2006). The nicking endonuclease (N.BstNB I) and 10 × NEBuffer 3 (50 mM Tris–HCl, 10 mM $MgCl_2$, 100 mM NaCl, pH 7.9) were obtained from New England BioLabs (Ipswich, MA, USA). All the other chemicals were of analytical grade and used as received. The synthesized oligonucleotides were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). All the oligonucleotides solutions were heat-treated in 90 °C for 3 min and then cooled in ice for 10 min prior to use. The sequences are listed as follows:

S1: 5′-NH_2-C6-TTT TTT T*CT GAC GCT GCT CAC G*-3′

S2: 5′-SH-C6-TTT TT*C GTG AGC AGC GTC AG*-3′ (The italic letters are complementary with the italic letters of S1)

Hairpin capture probe (CP): 5′-SH-C6-AAA GGG TTG GGC GG*G ATG GGT T*<u>**GA GTC**</u> **TTC C↓AG TGT GAT GA***A ACC CAT C*-3′ (The italic letters are the sequence of the stem arms; the underlined letters are the recognition sequence of N.BstNB I, and the arrow indicates the nicking position; the bold letters are complementary with the target DNA)

5′-NH_2-C6-AAA GGG TTG GGC GG*G ATG GGT T*<u>**GA GTC**</u> **TTC C↓AG TGT GAT GA***A ACC CAT C*-3′ for comparison biosensor fabrication

p53 gene target (TD): 5′-TCA TCA CAC TGG AAG ACT C-3′

Single-base mismatch target (sTD): 5′-TCA TCA CAC TGG AAG A<u>A</u>T C-3′

Non-complementary target (nTD):5′-GAC GTC AGA CTT CCT GCG A-3′.

2.2. Apparatus

CV and DPV were performed at room temperature (RT) on a CHI-660C electrochemical workstation (Chenhua Instrument Co., Ltd., Shanghai, China) coupled with a conventional three-electrode configuration where modified glassy carbon electrode (GCE, 3 mm in diameter) was used as working electrodes, platinum wire as an auxiliary electrode and saturated calomel as a reference electrode (SCE). The morphologies of materials were characterized by SEM (JSM-6700F) and TEM (JEM-2100). Absorption spectra were measured on a UV-2800A ultraviolet–visible (UV–vis) spectrophotometer (Unico Instrument Co., Ltd., Shanghai, China)

2.3. Synthesis of S2 and CP functionalized AuNPs

Initially, a mixture of 0.6 nM S2 and 2.4 nM CP was directly injected into 1000 μL gold colloids in a NaOH-treated glass vial (before conjugation, S2 and CP should be pretreated by TCEP to split the formed disulfide between the thiolated DNA probes). After gently shaking, the mixture was transferred to the refrigerator at 4 °C for further reaction (overnight). Then, added 10 μL 500 mM Tris–HCl (pH 7.4) and 115 μL 1 M NaCl dropwise