Biosensors and Bioelectronics 91 (2017) 183–189

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Ultrasensitive electrochemical DNA biosensor based on functionalized gold clusters/graphene nanohybrids coupling with exonuclease III-aided cascade target recycling

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ARTICLE INFO

Keywords:
Electrochemical biosensor
Target DNA
AuNCs/GR nanohybrids
Exonuclease III-aided cascade target recycling
Enzyme-catalyzed silver deposition

ABSTRACT

In this work, a novel and ultrasensitive electrochemical biosensor was constructed for DNA detection based on functionalized gold clusters/graphene nanohybrids (AuNCs/GR nanobybrids) and exonuclease III (Exo III)-aided cascade target recycling. By utilizing the capacity of GR as universal template, different metal nanoclusters including AuNCs/GR nanobybrids and PtNCs/GR nanohybrids were synthesized through convenient ultrasonic method. Exo III-aided cascade recycling was initiated by target DNA, generating the final cleavage product (S2), which acted as a linkage between capture probe and the functionalized metal nanoclusters/GR conjugates in the construction of the biosensor. The AuNCs/GR-DNA-enzyme conjugates acted as interfaces of enzyme-catalyzed silver deposition reaction, achieving DNA detection ranging from 0.02 fM to 20 pM with a detection limit of 0.057 fM. In addition, PtNCs/GR-DNA conjugates presented peroxidase-like activity and the functionalized PtNCs/GR nanohybrids-based electrochemical biosensor also realized DNA detection by catalyzing the 3,3′,5,5′-tetramethylbenzidine-hydrogen peroxide (TMB-H_2O_2) system to produce electrochemical signal. This metal clusters/GR-based multiple-amplified electrochemical biosensor provided an universal method for DNA detection.

1. Introduction

Nucleic acids are vital genetic material in cell nucleus which contain the instructions of genetic information. Besides, they are featured in protein biosynthesis, playing a decisive role in a series of major life process including growth, heredity and variation (Chargaff, 1951). Due to such a supernormal role, nucleic acids are specific biomarkers involved in many pressing diseases. Gene damage and gene mutation cause familial hereditary disease and intractable disease with high risk, for instance, myotonic muscular dystrophy, hemophilia, inflammatory bowel and tumour (Fu et al., 1992; Holzer, 1998; Kaghad et al., 1997; Levinson et al., 1987). Therefore, detection of trace amount of specific-sequence DNA is of hugely important in the early diagnosis of gene-related diseases, which is of signality in biomedical research and clinical diagnosis (Rosi et al., 2006; Zhang et al., 2013).

Detection methods for DNA include fluorescence (Zuo et al., 2010; Ye et al., 2016), surface-enhanced Raman scattering (SERS) (Ngo et al., 2016; Zeng et al., 2016), colorimetry (Liu et al., 2013; Yun et al., 2016) and electrochemical biosensor (Tan et al., 2015; Wang et al., 2016). There into, electrochemical biosensor based on diversified signal amplification strategies is an effective and sensitive tool for DNA detection (Gerasimova and Kolpashchikov, 2014; Zhao et al., 2015). In the construction of electrochemical biosensor, enzyme-catalyzed reaction and exonuclease (Exo)-aided target recycling have been widely used for signal enhancement, which improve the sensitivity of bioassays (Liu et al., 2016; Wang et al., 2015). In enzyme-catalyzed reaction, frequently-used enzymes are horse radish peroxidase (HRP), peroxidase, glucose oxidase (GOD) and alkaline phosphatase (ALP). Compared with other enzymes, ALP is arrestive for its activity in biocatalytic metal deposition reaction. In ALP-catalyzed silver deposition reaction, ascorbic acid 2-phosphate (AAP) is traditionally used as catalytic substrate, the generated ascorbic acid (AA) acts as reducing agent for Ag^+, the reduced Ag nanoparticles (AgNPs) finally deposited on the surface of ALP (Basnar et al., 2006; Feng et al., 2011). As to the Exo-aided target recycling, the presence of target initiates the cleavage process, the hydrolysis occurs upon target binding, followed with the releasing of target. Commonly used Exos are Exo I, RecJf, Exo III and λ-exo. Among them, Exo III is effective on 3′-terminal of double-

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http://dx.doi.org/10.1016/j.bios.2016.12.017
Received 1 September 2016; Received in revised form 5 December 2016; Accepted 7 December 2016
Available online 10 December 2016
0956-5663/

stranded DNA, thus we can flexibly utilize the cleavage property of Exo III to obtain a certain DNA sequence via appropriate design (Bao et al., 2015; Zhang et al., 2011).

Gold nanoclusters (AuNCs) are gold nanomaterial composed of several to few hundreds of Au atoms with ultra-small sizes commensurate to the Fermi wavelength of electrons. For the size-specific properties, AuNCs present distinctly different optical and chemical properties, which have attracted considerable attention and been widely used in many fields including fluorescence, photoluminescence and catalytic reactivity (Jin, 2015). So far, many methods have been applied to synthesize AuNCs. For example, thiolated compounds are used to reduce chloroauric acid to synthesize AuNCs, such as dithiothreitol, mercaptoundecanoic acid and 8-Mercapto-9-propyladenine (Ding et al., 2014; Sun et al., 2014; Venkatesh et al., 2014). Proteins also have been used as template to prepare AuNCs, including glutathione, trypsin and bovine serum albumin (BSA) (Farrag et al., 2013; Chang and Ho, 2015; Shamsipur et al., 2015). AuNCs also can be synthesized by the reductive effect of dimethylformamide (DMF) (Liu et al., 2008; Kawasaki et al., 2010). Besides, graphene (GR) can be used as template to synthesize AuNCs and obtain the AuNCs/GR nanohybrids (Yin et al., 2012). Compared with other methods, the GR-based method has short synthesis period without addition of protecting protein or reducing agent, which is very clean and simple. Morever, GR has incomparable conductibility compared with other templates including thiolated compounds and proteins. As to the AuNCs/GR nanohybrids, the conductive GR sheets act as effective mediator of the metal-support interaction, on which AuNCs distributed uniformly. Owing to the excellent conductivity of GR and AuNCs, the AuNCs/GR nanohybrids have promising potential in the construction of biosensing interfaces. Many DNA biosensors based on various nanomaterials and amplification strategies have been reported previously, including nitrogen-doped GR/Au nanoparticles, GR/Au nanorod/polythionine, DNAzyme amplification and Exo III-assisted cascade signal amplification (Chen et al., 2016; Huang et al., 2015; Liu et al., 2015; Xiong et al., 2017). However, they were limited by the complex synthetic procedures, expensive instrument or the low sensitivity. Compared with these reported biosensors, the superiority of the proposed biosensor was utilizing the GR as an universal template to synthesize different metal nanoclusters including AuNCs/GR and PtNCs/GR nanohybrids, which was very simple and convenient, and different catalytic interfaces and catalytic reactions were successfully constructed. The combination of several attractive amplification strategies including AuNCs/GR nanohybrids, Exo III-aided cascade target recycling and enzyme-catalyzed reaction improved the sensitivity significantly. Up to now, related research of AuNCs/GR nanohybrids-based functionalized catalytic interfaces coupling with Exo III-aided cascade target recycling for DNA recognition has not been reported.

Herein, we developed a multiple-amplified electrochemical biosensor for DNA detection based on functionalized AuNCs/GR nanohybrids and Exo III-aided cascade target recycling for signal amplification. Target DNA triggered the Exo III-aided cascade target recycling process which generated the final cleavage product (S2). With the presence of S2, the electrochemical biosensor was successfully constructed with the functionalized AuNCs/GR nanohybrids performing as the interfaces of enzyme-catalyzed silver deposition reaction. The proposed biosensor realized ultrasensitive DNA detection and could specifically distinguish target DNA from mismatched DNA. This strategy provided a versatile tool that had great potential for DNA detection in bio-analysis.

2. Experimental section

2.1. Materials and reagents

Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), silver nitrate ($AgNO_3$) and ALP were purchased from Sigma-Aldrich co. (St. Louis, USA). Ammonia solution, chloroplatinic acid hexahydrate ($H_2PtCl_6 \cdot 6H_2O$), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), cetyltrimethylammonium bromide (CTAB), albumin from bovine serum (BSA), glycine, sodium borohydride ($NaBH_4$), hydrogen peroxide (H_2O_2) and 3,3′,5,5′-tetramethylbenzidine (TMB) were received from Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). AAP was got from DiBai Chemical Technology co., Ltd. (Shanghai, China). All solutions used in the experiment were prepared using ultrapure water (resistivity of 18.25 MΩ cm) from Aquapro water purification system. All oligonucleotides were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China). HP1 and HP2 were heated to 90 °C for 10 min and slowly cooled to room temperature before use to form hairpin structure. The sequence of the oligonucleotides were as follows:

Hairpin DNA 1 (HP1): 5′-GACTCAGGCTTGATTAAGAGTCTTCC-3′;.

Hairpin DNA 2 (HP2): 5′-CAGGTACCTTAACGACTG CGAAGCCTGAGTC-3′;.

Target DNA: 5′-GGAAGACTCTTGTCT-3′;.

Assistance DNA: 5′-CGCAGTCGTTCTCA-3′;.

Capture probe: 5′-CGTTACGTCATGTGCTAAGGTACCTG-$(CH_2)_6$-SH-3′;.

SH-DNA: 5′-AGCACATGACGTAACG-$(CH_2)_6$-SH-3′;.

One-base mismatched DNA: 5′-GGAATACTCTTGTCT-3′;.

Two-base mismatched DNA: 5′-GGAATTCTCTTGTCT-3′;.

Noncomplementary DNA: 5′-ATCGCCAATCCAGGC-3′;.

2.2. Apparatus

Electrochemical experiments were measured with CHI 660E electrochemical workstation (CH Ins., Shanghai, China). The electrochemical detections were employed with a three-electrode system including gold electrode (GE) as working electrode, a Pt electrode as counter electrode and a saturated calomel electrode (SCE) as reference electrode. Transmission electron microscopy (TEM) images were observed with FEI Tecnai G20 transmission electron microscope (FEI company, Hillsboro, USA). UV–vis absorption spectra was recorded by UV-2700 UV–vis spectrophotometer (Shimadzu co., Ltd, Japan).

2.3. One-Step synthesis of AuNCs/GR nanohybrids

The AuNCs/GR nanohybrids were synthesized according to the method previously reported with slight modification (Yin et al., 2012). In a typical synthesis, 1.7 mg GR sheet was dissolved in 10 mL ammonia solution (pH =11, adjusted pH with 2 mM CTAB) and ultrasonically for 3 h to obtain GR solution (0.17 mg/mL). Then 100 μL $HAuCl_4$ (50 mM) was added into the above mixture and immediately ultrasonically for 10 min at room temperature with the power of 100 W. Whereafter, the obtained AuNCs/GR nanohybrids were centrifugated for 10 min at 10000 rpm and washed with ultrapure water thoroughly, then dispersed in 10 mL CTAB (2 mM).

2.4. Preparation of functionalized AuNCs/GR nanohybrids

AuNCs/GR nanohybrids were labeled with SH-DNA and ALP (AuNCs/GR-DNA-ALP) to construct the catalytic interfaces. AuNCs/GR-DNA-ALP conjugates were prepared as follows: 20 μL SH-DNA (1 μM) containing 2 mM TCEP was added into 1 mL AuNCs/GR nanohybrids and stirred for 2 h. Afterwards, 50 μL ALP (200 U/mL) with 2 μL NaOH (1 M) were introduced into the mixture and kept stirring for 3 h, and then 100 μL 1% BSA was added to blocked the unoccupied sites of AuNCs. The obtained AuNCs/GR-DNA-ALP conjugates were centrifugated for 10 min with 10000 rpm and washed with ultrapure water throughly, then dissolved in 10 mM phosphate buffer saline (PBS, pH 7.4) and stored at 4 °C for further use.