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Ultrasensitive electrochemical detection of nucleic acid by coupling an autonomous cascade target replication and enzyme/gold nanoparticle-based post-amplification

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ABSTRACT

Owing to the intrinsic importance of nucleic acid as bio-targets, the development of isothermal and ultrasensitive electrochemical DNA biosensor is very essential for biological studies and medical diagnostics. Herein, the autonomous cascade DNA replication strategy was effectively married with the enzyme/gold nanoparticle-based post-amplification strategy to promote the detection performance toward target DNA. A hairpin DNA probe (HP) is designed that consists of an overhang at 3′-end as the recognition unit for target DNA, a recognition site for nicking endonuclease, and an alkane spacer to terminate polymerization reaction. The autonomous DNA replication–scission–displacement reaction operated by the nicking endonuclease/KF polymerase induced the autocatalytic opening of HP, which was then specifically bound by the enzyme/gold nanoparticles for further dual-signal amplification toward target-related sensing events. A low detection limit of 0.065 fM with an excellent selectivity toward target DNA could be achieved. The proposed biosensor could be also easily regenerated for target detection. The developed biosensor creates an opportunity for the effective coupling of the target replication with post-amplification strategies and thus opens a promising avenue for the detection of nucleic acid with low abundance in bioanalysis and clinical biomedicine.

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1. Introduction

The development of DNA biosensors capable of amplified detection of DNA is being greatly motivated by its potential applications in clinical and diagnostic research (Enkin et al., 2014; Turner, 2013; Zhao et al., 2014; Zhang et al., 2013; Liang et al., 2014; Lin et al., 2015; Sawyers, 2008; Hsieh et al., 2012). Up to date, different isothermal signal amplification strategies have been proposed for the fabrication of DNA biosensors (Wang et al., 2014a; Yan et al., 2014; Lei and Ju, 2012). The well-developed signal amplification strategy can be simply categorized as two distinct types: post-amplification strategy toward the signal produced by hybridization or recognition event, and target recycling strategy. Examples of post-amplification strategy includes the use of enzymes (Liu et al., 2008a; Patolsky et al., 2001; Wilner et al., 2009; Shen et al., 2015), metal nanoparticles (Zhang et al., 2006; Song et al., 2010; Shi et al., 2015), DNA concatamer (Liu et al., 2014; Chen et al., 2012; Russell et al., 2014), DNA bio-barcode (Zhang et al., 2009; Song et al., 2014) as amplifying labels. The target recycling strategy is usually operated via nucleases for example exonuclease, endonuclease, polymerase or DNAzyme, to devote for the indirect amplification toward target number (Gerasimova and Kolpashchikov, 2014; Zuo et al., 2010; Xuan et al., 2012a, 2012b; Kong et al., 2011; Xu et al., 2012; Lu et al., 2012). Although great advances have been made toward the target detection by using each of these two strategies, the development of biosensor with further upgraded detection performance is still highly desirable to satisfy the requirement for the profiling trace amounts of biomarkers, and then serve for the clinical early diagnosis and treatment of some major diseases. It is conceived that the effective combination of these two classes of strategies would be attractive to address this issue. Unfortunately, it has been rarely reported for the biosensor fabrication via the grafting or marriage of these two strategies (Kong et al., 2014; Liu et al., 2013a; Wang et al., 2013; Chen et al., 2013). Recently, the nicking endonuclease-based target recycling has been ingeniously associated with rolling circle amplification (RCA)-based post-amplification for the development of ultrasensitive DNA biosensor (Ji et al., 2012; Wang et al., 2014b). But the assay is relatively complex, and also the biosensor can not

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be regenerated for the target DNA detection. The catalyzed hairpin DNA assembly (CHA)-based amplification strategy may be another choice for the coupling of target recycling and post-amplification (Yin et al., 2007; Qing et al., 2014; Jung and Ellington, 2014; Li et al., 2012). But the CHA strategy is usually restricted with the relatively rigor design principle for the adopted hairpin DNA. Recently, the development of DNA machine has attracted substantial concerns owing to its striking improvement for the detection performance toward target analytes (Wang et al., 2012; Lu et al., 2013; Yao et al., 2015; Duan et al., 2013; Weizmann et al., 2008). The most typical example is based on the autonomous replication–scission–displacement strategy by means of nicking endonuclease and polymerase (Yin et al., 2013; Zhuang et al., 2014; Freage et al., 2014; Weizmann et al., 2006; Wang et al., 2012; Chen et al., 2015). Accompanied with the DNA replication–scission–displacement process, the target analogs could be exponentially produced for the remarkable signal amplification. It thus holds a great promise to substitute PCR due to its easy operation, isothermal reaction, and high sensitivity. However, this autonomous DNA replication strategy is more preferentially connected with the fluorescence technique. By comparison, electrochemical method can provide significant advantages, such as its inherent signal stability, low cost, high sensitivity and ease of calibration (Xuan et al., 2012a, 2012b; Ren et al., 2014; Ronkainen et al., 2010; Rowe et al., 2011; Hu et al., 2014). Also, the instrumentation can be easily integrated with some miniaturized devices or lab-on-a-chip platforms. Considering the remarkable signal amplification capability of this machine-like DNA replication–scission–displacement strategy, it is therefore convinced that the effective coupling with the appropriate electrochemical signal readout or reporter system would offer a promising avenue for the ultrasensitive electrochemical detection of DNA.

Herein, the autonomous cascade DNA replication strategy was effectively married with the enzyme/gold nanoparticle-based post-amplification strategy for the first time to fabricate an ultrasensitive electrochemical DNA biosensor. A hairpin DNA probe (HP) is designed that consists of an overhang at 3′-end as the recognition unit for target DNA, a recognition site for nicking endonuclease, and an alkane spacer to terminate polymerization reaction. The assay protocol for target DNA mainly involves the following two modules: (1) autonomous DNA replication–scission–displacement module operated by the nicking endonuclease/KF polymerase for the indirect amplification toward target amounts and autocatalytic opening of HP to expose the caged DNA fragment in the stem region of the 5′-end of HP; (2) enzyme/gold nanoparticle-based dual-signal amplification module toward the above target-related sensing events. Also, this isothermal signal amplification reaction operated by the nicking endonuclease/KF polymerase involves no damage or cleavage toward the original probe DNA, indicating that it may be beneficial for the fabrication of the regenerated and amplified electrochemical DNA biosensor. The current proposed strategy creates an effective grafting for the target replication and post-amplification strategies and thus holds a huge potential for the detection of nucleic acid with low abundance in bioanalysis and clinical biomedicine.

2. Experimental section

2.1. Materials and chemicals

Tris(2-carboxyethyl)phosphine (TCEP), 6-Mercapto hexanol (MCH) and 1-Naphthyl phosphate (1-NP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nb.BbvCI endonuclease, Klenow Fragment (3′→5′ exo-) (KF polymerase), and deoxyribonucleoside triphosphates (dNTPs) were purchased from New England Biolabs, Inc. (Beverly, MA, USA). Bovine serum albumin (BSA) and streptavidin-alkaline phosphatase (SA-ALP) were obtained from Beijing Dingguo Biotechnology Company (Beijing, China). Fetal bovine serum was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). All of the other chemicals were of analytical reagent grade and used without further purification. The HPLC-purified oligonucleotide sequences are purchased from Sangon Biotech. Co., Ltd. (Shanghai, China) and listed in Table S1.

2.2. Apparatus.

All electrochemical measurements were carried out by using a CHI 660D electrochemical workstation (CH Instruments, Shanghai, China) at room temperature. A conventional three-electrode system was used, which comprised a gold working electrode (2 mm diameter), a platinum wire auxiliary electrode, and a Ag/AgCl reference electrode. TEM images were obtained using an FEI Tecnai (G2 20 TWIN) TEM at an acceleration voltage of 200 KV (FEI Company, USA). The particle size were measured using dynamic light scattering technique on a Malvern Zetasizer 3000 HS (Malvern Instruments, Worcestershire, UK).

2.3. Electrode pretreatment.

The gold electrode was cleaned by immersion in a freshly prepared piranha solution (a 3:1 v/v mixture of concentrated H_2SO_4 and 30% H_2O_2) for 20 min, followed by a thorough rinsing with ultrapure water. Then the electrode was polished on a microcloth (Shanghai Chenhua Inc., China) with 50 nm alumina slurry to obtain a mirror surface, followed by sonication in acetone and ultrapure water for 5 min each, to remove residual alumina powder. The well-polished electrode was then subjected to electrochemical pretreatment by cycling the potential between −0.2 and 1.5 V in H_2SO_4 (0.5 M) at a scan rate of 100 mV s^{-1} until a stable cyclic voltammogram was obtained, and then the cleaned electrode was allowed to be dried at room temperature.

2.4. Preparation of signal probe (SP) DNA conjugated gold nanoparticle (SP-AuNP).

The gold nanoparticles (AuNPs) with diameter of about 13 nm were prepared as reported previously (Grabar et al., 1995). Simply, after heating 100 mL of 1 mM $HAuCl_4$ solution to 100 °C, 10 mL of 38.8 mM trisodium citrate was added quickly to the boiling solution under continuous stirring. The reaction mixture was stirred at 100 °C for 15 min until the color turned red and then stored at 4 °C. A volume of 50 μL biotin-labeled signal probe DNA (SP1 DNA) (10 μM) was then added to 1 mL of AuNPs solution and stirred at room temperature overnight. Afterward, 0.1 mL of PBS containing 2 M NaCl was added to the mixture stepwise for stabilizing the obtained AuNPs probe, which was centrifuged at 10,000 rpm and washed with PBS twice, and then resuspensed in 0.5 mL of PBS (Qian et al., 2014). The SP1 DNA conjugated AuNPs (SP1-AuNPs) were characterized by UV–vis spectra (Fig. S1 in the Supporting Information). The characteristic absorption peak of AuNPs was red-shifted from 521 to 525 nm due to the decoration of SP1 DNA on AuNPs (Wang et al., 2014c). It could be also seen from Fig. S2 that the average hydrodynamic size for SP1-AuNPs was evidently increased than that of the bare AuNPs. The increased size came from the conjugated SP1 DNA on the AuNPs (Wang et al., 2015; Zheng et al., 2013). Furthermore, the obtained AuNPs and SP1-AuNPs were also characterized by TEM techniques (Fig. S3). No evident change about the size and morphology for each SP1-AuNP could be observed compared with that of the bare AuNP.

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