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A cascade autocatalytic strand displacement amplification and hybridization chain reaction event for label-free and ultrasensitive electrochemical nucleic acid biosensing

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ABSTRACT

Herein, an autocatalytic strand displacement amplification (ASDA) strategy was proposed for the first time, which was further ingeniously coupled with hybridization chain reaction (HCR) event for the isothermal, label-free and multiple amplification toward nucleic acid detection. During the ASDA module, the target recognition opens the immobilized hairpin probe (IP) and initiates the annealing of the auxiliary DNA strand (AS) with the opened IP for the successive polymerization and nicking reaction in the presence of DNA polymerase and nicking endonuclease. This induces the target recycling and generation of a large amount of intermediate DNA sequences, which can be used as target analogy to execute the autocatalytic strand displacement amplification. Simultaneously, the introduced AS strand can propagate the HCR between two hairpins (H1 and H2) to form a linear DNA concatamer with cytosine (C)-rich loop region, which can facilitate the in-situ synthesis of silver nanoclusters (AgNCs) as electrochemical tags for further amplification toward target responses. With current cascade ASDA and HCR strategy, the detection of target DNA could be achieved with a low detection limit of about 0.16 fM and a good selectivity. The developed biosensor also exhibits the distinct advantages of flexibility and simplicity in probe design and biosensor fabrication, and label-free electrochemical detection, thus opens a promising avenue for the detection of nucleic acid with low abundance in bioanalysis and clinical biomedicine.

1. Introduction

The development of nucleic acid biosensor is of great importance for clinical diagnosis, food analysis, environmental monitoring, and bioterrorism (Jung and Ellington, 2014; Du and Dong, 2017; Liu et al., 2009; Smith et al., 2017). Over the past decades, different techniques such as electrochemistry, spectroscopy, surface plasmon resonance, colorimetry, and so on, have been well explored for DNA biosensor fabrication (Baaske et al., 2014; Schena et al., 1995; Zhang et al., 2017; Zhao et al., 2014; Shu et al., 2018; Qiu et al., 2017b). Among them, electrochemical method shows some prominent features including inherent signal stability, low cost, ease of calibration and miniaturization (Das et al., 2015; Drummond et al., 2003; Xiao et al., 2007; Qiu et al., 2018). Currently, the polymerase chain reaction should be the most often employed method for the amplified analysis of nucleic acid (Hindson et al., 2013; Heid et al., 1996). However it is often limited by the delicate control of temperature cycling and complex primer design. Thus the pursuit of isothermal amplification strategy for the profiling of trace amounts of nucleic acid is in high demand.

Till now, different isothermal amplification methods have been advocated for nucleic acid biosensor fabrication, which further include nuclease-based or enzyme-free strategy based on the use or not of protein enzyme (Zhao et al., 2015; Craw and Balachandran, 2012; Chen et al., 2017; Zhang et al., 2018; Qiu et al., 2017a). The nuclease-based signal amplification is usually operated by the individual or combined role of various nucleases including endonuclease, exonuclease and polymerase, etc. to contribute the signal amplification via target recycling operation (Wang et al., 2011; Guo et al., 2018; Liu et al., 2015). Especially, the autonomous replication-scission-displacement strategy by means of polymerase and nicking endonuclease could afford an isothermal exponential amplification capability toward nucleic acid detection (Weizmann et al., 2006; Chen et al., 2015; Zhang and Zhang, 2012; Yin et al., 2013; Nie et al., 2015). However, a linear DNA template is commonly used, which is not beneficial for the selective discrimination of target DNA, especially for base mismatch analysis. Also the generated DNA replicates could hybridize with the free DNA template, restricting the detection performance toward target to some extent. Furthermore, it is more prone to the fluorescence detection. Thus

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upgradation of such isothermal exponential amplification system to improve its detection sensitivity and selectivity, and adaptability with electrochemical measurement remains highly desirable. The enzyme-free amplification strategy could be regarded as another research focus for nucleic acid biosensor fabrication, which is usually designed on the basis of dynamic DNA assembly principle (Zhang and Seelig, 2011; Liu et al., 2017). The typical examples include catalytic hairpin DNA assembly (CHA) and hybridization chain reaction (HCR) strategies. The CHA is operated with the catalytic assembly of two hairpins into duplex DNA in the presence of target trigger for signal amplification (Yin et al., 2008; Li et al., 2011). However, it is often confronted with the relatively rigorous sequence design, which would easily induce a relatively high background signal owing to the potentially non-specific reaction between two hairpin substrates. The HCR is operated with the alternate assembly of two DNA hairpins in the presence of target to form a DNA concatamer. It can be used as an universal post-amplification strategy for the fabrication of various biosensors toward the analysis of nucleic acid, protein and small molecules (Ikbal et al., 2015; Dirks and Pierce, 2004; Ge et al., 2018; Zhou et al., 2018). Although these substantial advances have been made for the fabrication of amplified nucleic acid biosensors, the development of simple, isothermal and autonomous amplification system with further upgraded detection performance is still highly demanded to satisfy the requirement for the profiling of trace amounts of biomarkers.

Herein, an autocatalytic strand displacement amplification (ASDA) was firstly proposed via the combined role of nicking endonuclease (Nt.BbvCI) and Bst DNA polymerase, which was then ingeniously grafted with hybridization chain reaction (HCR)-based post-amplification strategy for the fabrication of an isothermal, ultrasensitive and label-free electrochemical nucleic acid biosensor. The developed sensing strategy includes two tandem and autonomous modules: ASDA and HCR. During the ASDA module, the target recognition with the immobilized hairpin probe (IP) on the electrode initiates the annealing of the auxiliary DNA strand (AS) with the opened IP. Then the polymerization and nicking reaction proceeds for the target recycling and generation of a large amount of intermediate DNA sequences, which could be used as target analogy to contribute the autocatalytic amplification toward target recognition events. Simultaneously, the introduced AS strand can propagate the HCR between two hairpins (H1 and H2) to form a linear DNA concatamer with cytosine (C)-rich loop region, which can facilitate the in-situ synthesis of silver nanoclusters (AgNCs) as electrochemical tags for further amplified detection. The AgNCs synthesized by C-rich loop DNA template have been reported for the fluorescent and electrochemical detection of various biomolecules (Enkin et al., 2014; Liu et al., 2013; Dong et al., 2012; Zhang et al., 2013; Yang et al., 2015; Orbach et al., 2013). With current cascade ASDA and HCR amplification strategy, the amount of in-situ synthesized AgNCs is dramatically enhanced, inducing substantially amplified current response for label-free and ultrasensitive detection of nucleic acid down to 0.16 fM.

2. Experimental section

2.1. Chemicals and materials

The nicking enzyme Nt.BbvCI (It can recognize the specific nucleotide sequence of 5′-CC↓TCAGC-3′ in a dsDNA and cut the nucleotide sequence in the arrow), Bst DNA polymerase (Large Fragment) and 10 × NEB buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9) were purchased from New England Biolabs Inc. (Ipswich, MA, USA). The deoxyribonucleoside 5′-triphosphate (dNTPs) mixture, tris(2-carboxyethyl) phosphine hydrochloride (TCEP) and fetal calf serum were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). 6-Mercapto-1-hexanol (MCH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Silver nitrate ($AgNO_3$) was purchased from Aladdin Reagents Inc. (Shanghai, China). Acrylamide/bisacrylamide 39:1 40% gel stock solution, N,N,N′,N′-tetramethylethylenediamine (TEMED), and ammonium persulfate (APS) were purchased from Yantai Science and Biotechnology Co., Ltd. (Yantai, China). T safeTM dye was purchased from Shanghai Tianneng Science and Technology Co., Ltd. (Shanghai, China). The HPLC-purified DNA sequences were synthesized by Sangon Biotech. Co., Ltd. (Shanghai, China) and listed below. The immobilized hairpin probe (IP): 5′-SH-$(CH_2)_6$-TTTCCACTCTCGCAGAGACCGGCGCACAGAGGCTG AGGCGAGAGTGGTTT-3′; Target DNA (TD): 5′-TTCCTCTGTGCGCCGG TCTCTCCT-3′; One-base mismatched DNA (1MT): 5′-TTCCTCTGTGCG CCAGTCTCTCCT-3′; Three-base mismatched DNA (3MT): 5′-TTCCTCT GTGCGAGAGTCTCTCCT-3′; Non-complementary DNA (NC): 5′-CAAG AAATTTTTTCTCCGGGTCAC-3′; Auxiliary DNA strand (AS): 5′-TCAGC TGATCAGCCCACTCTCGC-3′; Target DNA2 (TD2) (It was designed to directly hybridize with IP and trigger the hybridization chain reaction event): 5′-TCA GCT GAT CAG CCC ACT CTC GCC TCA GCCTCTGTGC GCCGGTCTCT-3′; Hairpin DNA1 (H1): 5′- GCTGATCAGCCCCCCCCCC CCCTGATCTGCATCTAGAT-3′; Hairpin DNA2 (H2): 5′-TCAGCTGATCA GCATCTCCCCCCCCCCCCAGATGCAGA-3′; H1 without the C-rich region: 5′- GCTGATCAGTTAGATTAGATTCTGATCTGCATCTAGAT-3′; H2 without the C-rich region: 5′-TCAGCTGATCAGCATCTTTAGATT AGAT TAGATGCAGA-3′. All other reagents were of analytical grade without further purification. The used target DNA and mutated sequence is related with a tumor suppressor gene, P53 exon8 DNA. The P53 gene is a crucial biomarker in early cancer diagnosis and treatment, and known as “the guardian of gene” (Ko and Prives, 1996; Batchelor et al., 2008). All solutions were prepared with deionized water obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA) with a resistivity of 18.2 MΩ cm.

2.2. DNA immobilization on the electrode surface

The gold electrode (2 mm diameter) was sequentially polished with 0.3 and 0.05 μm alumina slurries to obtain a mirror surface, followed by sonication in ethanol and deionized water for 5 min, respectively, to remove the residual alumina powder. Then, the electrode was electrochemically scanned in 0.5 M H_2SO_4 solution within a potential window between − 0.2 and + 1.5 V at a scan rate of 100 mV/s till a stable cyclic voltammogram was obtained. All the DNA sequences were heated to 90 °C for 5 min and then allowed to cool to room temperature for at least 2 h before use. The treated electrode was incubated in 50 μL immobilization buffer (20 mM Tris-HCl, 0.1 M NaCl, 10 mM TCEP, pH 7.4) containing 1 μM IP at room temperature for 4 h (Other concentrations of IP including 0.1, 0.5, 2.0 and 5.0 μM were also studied for the immobilization optimization). Then the resulting IP modified electrode was blocked with 1 mM MCH solution for 30 min to remove the nonspecific adsorbed DNA.

2.3. Autocatalytic strand displacement amplification and hybridization chain reaction

The IP modified electrode was incubated with 50 μL 1 × NEB buffer containing 1 μM auxiliary strand (AS), 10 U nicking endonuclease, 8 U Bst polymerase, 200 μM dNTPs and various concentrations of target DNA for 80 min at 37 °C. Followed by washing with 10 mM Tris-HCl (500 mM NaCl, 1 mM $MgCl_2$, pH 7.4), the electrode was incubated into the mixture of H1 (0.5 μM) and H2 (0.5 μM) in 10 mM Tris-HCl (500 mM NaCl, 1 mM $MgCl_2$, pH 7.4) for 2.5 h at room temperature. The sensors were then washed with Tris-HCl buffer and dried. Afterward, 10 μL of $AgNO_3$ solution (100 μM) in citrate buffer (20 mM sodium citrate, pH 7.0) was dropped on the electrode surface for 20 min in dark. Subsequently, 2 μL of fresh $NaBH_4$ solution (500 μM) in citrate buffer was added on the electrode surface at room temperature for 1 h in dark to reduce $AgNO_3$ to AgNCs.