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Short Communication

Electrochemical biosensor for DNA demethylase detection based on demethylation triggered endonuclease BstUI and Exonuclease III digestion

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A B S T R A C T

Herein, an electrochemical biosensor was fabricated for DNA demethylase detection based on DNA demethylation triggered endonuclease BstUI and Exonuclease III digestion. After the double-strand DNA was demethylated, it can be further digested by BstUI and formed a blunt end at the electrode surface. Then, the remained fragment of DNA–DNA duplex was further cleaved by exonuclease III and led to increased electrochemical signal. Based on this detection strategy, the biosensor showed high sensitivity with low detection limit of 0.15 ng/mL. Moreover, the developed method also presented high selectivity and acceptable reproducibility. This work provides a novel detection platform for DNA demethylase detection.

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1. Introduction

DNA methylation is an important epigenetic modification and plays crucial role in differential control of gene expression (Siegfried and Cedar, 1997). It is catalyzed by DNA methyltransferase (MTase), which can transfer one methyl group from the donor of *S*-adenosyl-*L*-methionine (SAM) to the C-5 position of cytosine, and produces 5-methylcytosine (5-mC) and *S*-adenosyl-*L*-homocysteine (SAH) (Horton et al., 2006). Due to the correlation of many diseases with the aberrant expression of DNA MTase, DNA methylation and DNA MTase activity have been considered as potential biomarkers for diseases diagnosis. Though DNA methylation has ever been supposed as a kind of stable epigenetic modification, DNA demethylation process also exists, which is catalyzed by DNA demethylase to remove the methyl from 5-mC (Bhattacharya et al., 1999).

As the opposite process of DNA methylation, DNA demethylation also plays vital roles in gene epigenetic regulation and involves in many diseases, such as cancers, imprinting-related diseases, and psychiatric disorders (Suzuki et al., 2006; Tsankova et al. 2007; Wang et al., 2012). Therefore, it is crucial to develop a sensitive method for the assessment of DNA demethylase activity. However, for this assay, the difficulties came from not only the low abundance of DNA demethylase, but also possible intereference, such as some proteins coexisting with DNA demethylase (Lopez-

Serra et al., 2006). Unlike the wide researches on assaying the activity of DNA MTase, little work has been done for detecting DNA demethylase. Recently, enzymatic control of plasmonic coupling as a surface enhanced Raman scattering (SERS) nanosensor was fabricated for DNA demethylation detection and DNA demethylase assay (Wang et al., 2012). After the hemimethylated DNA probes were demethylated, the degradation reaction of the probes methylation-sensitive endonuclease Bsh1236I and single-strand selective exonuclease I was triggered, which resulted in the aggregation of gold nanoparticles (AuNPs) and generated strong plasmonic coupling SERS signal in response to DNA demethylation. This work showed the advantages of instrument miniaturization, low background signal, excellent specificity, convenient, and reproducible detection with homogeneous, single-step operation. However, this method suffers from expensive and large-volume instruments for signal detection, which might limit its applicability.

Electrochemical methods have been demonstrated their virtues on DNA methylation detection and DNA MTase activity assay with the advantages of simple operation, easy preparation, low cost, fast response, high sensitivity and selectivity (Deng et al., 2014; He et al., 2011; Li et al., 2012; Liu et al., 2011; Sato et al., 2006; Sato et al., 2012; Su et al., 2012; Wang et al., 2013; Yin et al., 2013a). Thus electrochemical techniques should also be a suitable detection platform for the assay of DNA demethylase activity. Herein, we develop a simple and sensitive electrochemical method for DNA demethylase detection based on its catalytic demethylation activity and demethylation trigged endonuclease BstUI–Exonuclease III

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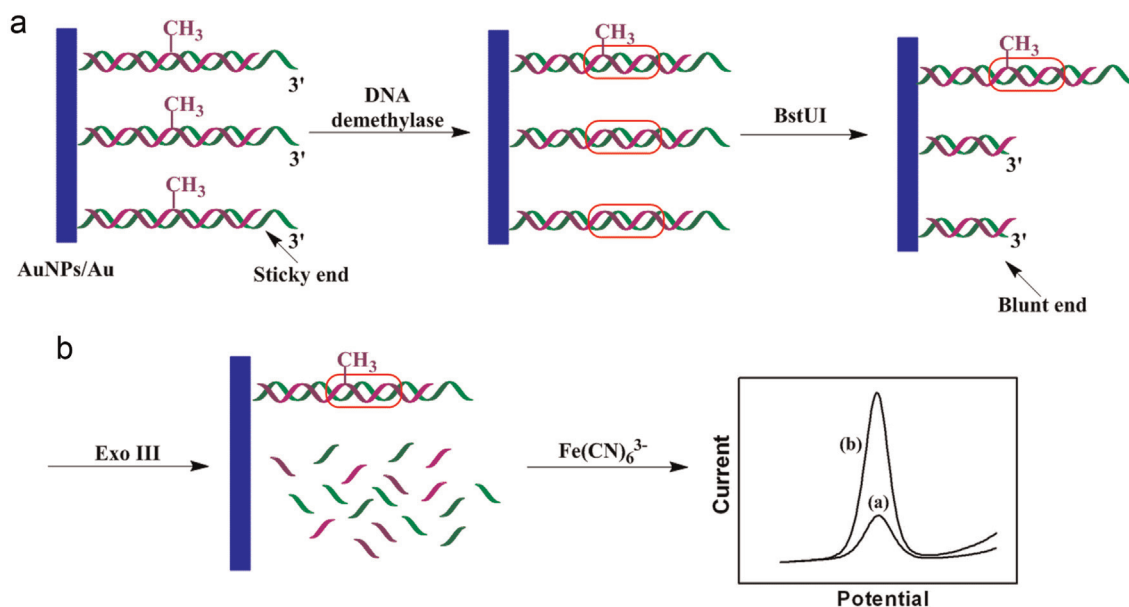
Scheme 1. Illustration of DNA demethylase detection based on electrochemical biosensor.

digestion, where Exonuclease III has been demonstrated its application in DNA detection (Fan et al., 2012; Luo et al., 2013). The detection mechanism is shown in Scheme 1. For improving the electrode effective surface and conductivity, the bare Au electrode is first modified with gold particles (AuPs) through electrochemical deposition under constant potential. Then, the DNA probe S1 is assembled on AuPs/Au surface through the Au–S bond and hybridized with the cytosine-methylated complementary DNA S2, forming hemi-methylated double-strand DNA hybrids, which contain the common recognition sequences (5′-CGCG-3′) for DNA demethylase and endonuclease BstUI (only digesting unmethylated sequence). Without demethylation, the BstUI activity is inhibited due to the cytosine methylation in 5′-CGCG-3′ sequence. Moreover, the formed S1–S2 hybrids contain a 3′-protrusion sticky end, which blocks the digestion activity of Exonuclease III because it has 3′ to 5′ exodeoxyribonuclease activity, which is specific for double-stranded DNA (Henikoff, 1984). Thus, at this state, the S2–S1/AuPs/Au cannot be digested by BstUI–Exonuclease III system, which causes a low electrochemical reduction current due to the stronger electrostatic repulsion effect between the negative charge controlled phosphate backbones of the oligonucleotides and the redox probe of $[Fe(CN)_6]^{3-}$ (Sharifi et al., 2014). However, when the S2–S1 hybrids are demethylated by DNA demethylase, they can be further cleaved by BstUI at the middle of 5′-CG|CG-3′ and form a blunt end at the electrode surface. Afterwards, those residual S2–S1 hybrids can be further digested by Exonuclease III, which will greatly decrease the electrostatic repulsion effect and result in a high electrochemical signal. Based on it, a signal "on" electrochemical method can be developed for DNA demethylase detection.

2. Experimental

2.1. Reagents and instruments

DNA demethylase and MBD4 protein were purchased from Epigentek Group Inc. (Farmingdale, NY, USA). MBD1 and MeCP2 protein were expressed and purified as described in our previous works (Yin et al., 2013a; Yin et al., 2013b). BstUI and Exonuclease III were obtained from New England Biolabs (USA). Tris(hydroxymethyl)aminomethane (Tris), EDTA and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Aladdin

(Shanghai, China). All DNA sequences were supplied by Sangon Biotech Co., Ltd. (Shanghai, China). The oligonucleotides sequences were selected randomly and shown as follows. DNA probe S1, 5′-SH–(CH$_2$)$_6$–TTC ACC TGT GTT TTT TCA T*GC GCG* AGA TCC CCC-3′, hemi-methylated complementary DNA (DNA S2), 5′-TCT C̲^{m}*GC G*CA TGA AAA AAC ACA GGT GAA-3′.

The buffer solutions employed in this work are as follows. Probe immobilization buffer, 10 mM Tris–HCl, 1.0 mM EDTA, 1.0 M NaCl and 1.0 mM TCEP (pH 7.4). DNA hybridization buffer, 10 mM Tris–HCl, 1.0 mM EDTA, and 1.0 M NaCl (pH 7.4). DNA demethylase buffer, 10 mM Tris–HCl, 200 mM NaCl, and 10 mM MgCl$_2$ (pH 7.5). Exo III reaction buffer, 10 mM Tris–HCl, 10 mM MgCl$_2$, and 1 mM DTT (pH 7.0). BstUI reaction buffer, 10 mM Tris–HCl, 10 mM MgCl$_2$, 100 mM KCl, and 0.1 mg/mL BSA (pH 8.5). Electrochemistry determination buffer, 10 mM PBS containing 5 mM $[Fe(CN)_6]^{3-}$ and 0.1 M KCl (pH 7.4). Washing buffer, and 10 mM Tris–HCl (pH 7.4).

Differential pulse voltammetry (DPV) was performed at CHI830D electrochemical workstation (CH Instrument, Inc. Austin, USA) in the potential range of -0.2–0.6 V. The parameters of increment potential, 0.004 V; pulse amplitude, 0.05 V; pulse width, 0.05 s; sample width, 0.0167 s; pulse period, 0.2 s; and quiet time, 2 s. The plane Au ($d=2$ mm, geometric area is 0.0314 cm^2, Gaoss Union®, China) or modified Au electrode was used as the working electrode, saturated calomel electrode as the reference electrode and platinum wire as the counter electrode.

2.2. DNA immobilization and hybridization

The plane gold electrode was firstly polished to mirror-like surface using 0.3 μm alumina slurry, followed with ultrasonic cleaning in double-distilled deionized water and anhydrous alcohol for 3 min. The gold electrode was rinsed with double-distilled deionized water and dried under N$_2$ blowing. Then the gold electrode was immersed into 3 mM HAuCl$_4$ solution containing 0.1 M KNO$_3$ and the AuPs were electrochemically deposited on the electrode surface using single-potential mode at -0.2 V for 200 s under stirring. The morphology of this electrode (AuPs/Au) was characterized by SEM (Supplementary materials). Afterwards, the gold electrode was incubated with 5 μL of probe immobilization buffer containing 0.5 μM probe DNA S1 for 12 h at ambient temperature under humid environment. The electrode was rinsed with washing buffer three times and noted as S1/AuPs/Au.