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Ultrasensitively electrochemical detection activity of DNA methyltransferase using an autocatalytic and recycling amplification strategy

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ABSTRACT

Herein, we report a novel approach to the ultrasensitive detection of DNA methyltransferase (MTase) in terms of a dual-signal recycling amplification strategy assisted by an autocatalytic and exonuclease III (Exo III). A duplex DNA probe was designed by G-quadruplex-forming oligomer (S1) hybridizing with ferrocene (Fc)-labeled DNA (S2) modified on Au electrode. With DNA adenine methylation (Dam) MTase, the response of the hairpin probe to methylation is methylated. Then it can be cleaved by the methylation-sensitive restriction endonuclease Dpn I, releasing S3. The released S3 sequence hybridizes with the dangling end of S2 sequence, forming a new duplex DNA. Then the Exo III can cleave the S2 sequence step by step, with the release of Fc from the sensing interface and autonomous generation of new secondary S3 for successive hybridization and cleavage. Meanwhile, S1 sequences on the electrode surface are folded into G-quadruplex-hemin complexes by adding K^+ and hemin to give a remarkable electrochemical response. Such conformational changes result in the decrease of differential pulse voltammetry (DPV) peak current of Fc and increase of the DPV peak current of G-quadruplex-hemin complexes. The dual-signal changes are linear with the concentration of DNA MTase. This novel, single electrode-based dual-signal assay strategy is superior to previous Dam assays using single electrochemical signal as output.

1. Introduction

DNA methylation catalyzed by methylase plays an important role in cell proliferation, genomic stability, and senescence in both eukaryotes and prokaryotes [1,2]. The process is implemented when DNA methyltransferases (MTases) transfer a methyl group from S-adenosyl-L-methionine (SAM) to cytosine or adenine bases [3]. Aberrant DNA methylation would cause tumor suppressor genes inactivation and transcriptional silencing gene expression [4]. Importantly, changes in DNA MTase activity generally occur long before other marks of cancer [5]. Therefore, the level of methylation is closely associated with DNA MTase activity [6–8]. Recent reports have demonstrated that DNA MTase may become a potential biomarker to clinical diagnostics and therapeutics [9–11]. Highly sensitive, accurate detection of DNA MTase activity would likely lead to equally accurate diagnosis of genetic disease. MTase activity assays based on HPLC [12,13], mess spectrum [14,15], colorimetric methods [16,17], real-time PCR-based methylation-specific PCR [18], electrogenerated chemiluminescence [19], and fluorescence methods [20] have been reported. Although these approaches have made significant contributions to the detection of MTase activity, their application is time-consuming, labor-intensive, complicated and expensive. This calls for developing simple assay for MTase activity with high sensitivity and selectivity.

Electrochemical biosensors have attracted substantial attention owing to their simple instrumentation, high sensitivity, and miniaturization [21–23]. These techniques are promising alternative to colorimetric and fluorescence assays in detecting DNA MTase activity [24]. Liu et al. reported an electrochemical assay to detect MTase activity and inhibitor screening based on a ferrocene (Fc) acetic acid-labeled DNA probe coupled with HpaII endonuclease [25]. Still another electrochemical method employs methylene blue (MB)-conjugated DNA as a response probe [26]. The reported electrochemical methods mainly rely on the target response signal from only one DNA strand of the DNA duplex, while the response signal from another DNA strand is not used reasonably. In reality, utility of response signals from electroactive labeled duplex DNA strands can improve the sensitivity and selectivity of electrochemical biosensors. While some studies have reported the multiplexed sensors utilizing two redox species to detect two different analytes [27], superimposing dual-signal change as an amplification strategy in the detection of DNA MTases has not been reported. Herein,

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we take advantage of DNA sequences which can be folded into G-quadruplex structures [28], coupling with hemin and the Fc-labeled DNA as two independent signals to detect DNA MTases with the help of Exo III [29].

In this study, we construct a novel, single electrode-based, dual-signal biosensor strategy for DNA Mtases detection based on an autocatalytic and Exo III-assisted recycling strategy. In the presence of Dam, the hairpin probe response to methylation is methylated. And then it can be cleaved by the methylation-sensitive restriction endonuclease Dpn I and releasing S3. The released S3 sequence hybridizes with the dangling end of S2 sequence, forming a new duplex DNA. Then the Exo III can cleave the S2 sequence step by step, with the release of Fc from the sensing interface and autonomous generation of new secondary S3 for successive hybridization and cleavage. Such conformational changes result in the decrease of differential pulse voltammetry (DPV) peak current of Fc and increase of the DPV peak current of G-quadruplex-hemin complexes. These designs together allow a high sensitivity for the Dam. In addition, this method can be further applied to anticancer drugs discovery.

2. Experimental section

2.1. Materials

Dam MTase, exonuclease III, S-adenosyl-L-methionine (SAM), NT.ALWI nicking endonuclease, Dpn I endonuclease and DL-dithiothreitol (DTT) were purchased from New England Biolabs Ltd. (Beijing, China). All DNA involved in this work were obtained from Sangon Biotech. Co., Ltd. (Shanghai, China), the detailed information was listed:

The quadruplex-forming oligomer S1:

5′-CACTGGGTTGGGCGGGATGGGTTTTTT $(CH_2)_6$-SH-3′,

Fc-labeled DNA sequence S2:

5′-Fc-$(CH_2)_6$-ATCCCGCCCAACCCAGTGAAGTTGGGA-3′,

The hairpin DNA:

5′-GTTGGGATCGAGAAGTTTTTCTTCTCGATCCCAACTTTGTCT-3′.

All other chemicals were of analytical grade. The water used for all experiments was ultrapure water (~18.2 MΩ).

2.2. Electrode pretreatment

Before using the gold electrode, it has to be underwent the next process. Firstly, the gold electrode was immersed in a freshly prepared piranha solution for 20 min, and then rinsed thoroughly with ultrapure water. Next, using alumina slurry (50 nm), the gold electrode was polished with the microfiber cleaning cloth to obtain a mirror surface, and then sonicated with acetone and ultrapure water sequentially to remove residual alumina slurry. Finally, the well-polished electrode was immersed in 0.5 M H_2SO_4 solution by cycling the potential between − 0.2 and 1.5 V under a scan rate of 100 mV s^{-1}. After a stable cyclic voltammogram was observed, the gold electrode was cleaned with ultrapure water and dried at room temperature [30].

2.3. Oligonucleotide immobilized on gold electrode

After the quadruplex-forming oligomer (S1) was pretreated with DTT for 2 h, an illustra™ NAP-5 column was used to remove the DTT. The pretreated Au electrode was immersed in buffer (10 mM Tris-HCl, 100 mM NaCl) containing S1 DNA (1.0 μM) for 16 h, then thoroughly rinsed with buffer. In water bath at 37 °C, the DNA-modified electrode was then immersed in 1.0 μM S2 DNA solution for 3 h.

2.4. DNA methylation and cleavage of Dpn I

The methylation experiment was performed in 40 μL of methylase buffer (10 mM Tris-HCl pH 7.4, 10 mM NaCl, 10 mM EDTA, and 1 mM DTT) containing 80 μM SAM, various amounts of Dam MTase and 1 μM hairpin probe. The reaction mixture was incubated for 4 h at 37 °C, followed by inactivation for 10 min at 65 °C. The mixture was then incubated with Dpn I at 37 °C for 3 h for digestion reaction.

2.5. Exo III-assisted autocatalytic recycling

The obtained DNA duplex probe-modified electrode was then immersed in 50 μL Tris-HCl solution (10 mM, pH 7.4) containing 100 mM NaCl, 10 mM $MgCl_2$, 5 units/μL Exo III and the cleaved products at 37 °C for 2 h. After thoroughly rinsed, the resulting electrode was then incubated with 2 μM hemin for 30 min in 10 mM HEPES buffer (pH 8.0, 50 mM KCl, 1% DMSO) to induce the liberated S1 folding into a G-quadruplex–hemin complex.

2.6. Electrochemical measurement

DPV was performed on a CHI 630C workstation (three-electrode system: a modified Au electrode as the working electrode, an Ag/AgCl electrode as the reference, and a platinum wire as the auxiliary) and recorded from − 0.6 to 0.6 V in 10 mM HEPES buffer (pH 8.0, 10 mM KCl). EIS was carried out in 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution from 0.01 Hz to 10 kHz on an Autolab PGSTAT30 electrochemical workstation.

2.7. Inhibition assay

Inhibition effect can also be quantitatively analyzed using the ratios of $I_{Fc}/I_{G\text{-}DNA}$. Different concentrations of 5-fluorouracil were mixed with 1 μM hairpin DNA in Dam buffer (10 mM Tris-HCl, pH 7.4, 1 mM DTT, 10 mM EDTA, and 10 mM NaCl), followed by addition of 40 units/mL Dam MTase and 80 μM SAM and then incubation at 37 °C for 4 h. The mixture was then incubated with Dpn I at 37 °C for 3 h for digestion reaction. The DNA duplex probe-modified gold electrode was then incubated with the resulting solution and treated with 5 units/mL Exo III for 2 h at 37 °C. After rinsed thoroughly, the electrode was incubated with 2 μM hemin in HEPES buffer (10 mM), DPV peak current intensity was recorded using the aforementioned procedure.

2.8. Assay of methylation by gel electrophoresis

Gel electrophoresis images were scanned by the Gel Image Analysis System (Bioshine GelX1650, China). In this test, 10 μL sample (1 μM hairpin DNA, 80 μM SAM, 5 units/mL Exo III, 40 units of Dam MTase, 80 units/mL Dpn I, 2 μM (S1 + S2) DNA, 10 mM Tris-HCl, 100 mM NaCl, and 10 mM $MgCl_2$, pH 7.4) was incubated at 37 °C, then put on a polyacrylamide gel to separate the cleaved products from the substrate. Electrophoresis was carried out at 110 V constant voltage for 3 h. After ethidium bromide staining, gels were photographed by a gel imaging system.

3. Results and discussion

3.1. Dam MTase activity assay principle

Scheme 1 illustrates the method for the detection of Dam MTase. As shown in Scheme 1, this strategy includes three main processes: (1) a hairpin DNA probe is methylated by Dam MTase and cleaved by Dpn I, (2) Exo III-assisted autocatalytic recycling amplification, and (3) electrochemical measurement. In the first step, the hairpin DNA probe containing sequence of 5′-G-A-T-C-3′ in the stem part is methylated by Dam MTase and cleaved into three parts by Dpn I in the sequence 5′-G-A-T-C-3′. Part one is a 21-base hairpin DNA containing a loop, part two is a 5-base blunt terminus, and part three is a 14-base S3. In the second and third steps, the Fc-labeled DNA probe (S2) is designed to hybridize with the thiolated G-quadruplex oligomer (S1) immobilized on Au