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Highly sensitive homogeneous electrochemical assay for methyltransferase activity based on methylation-responsive exonuclease III-assisted signal amplification

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ABSTRACT

DNA methylation catalyzed by methyltransferase (MTase) plays a critical role in many biological processes. In this paper, a novel and highly sensitive homogeneous electrochemical assay was developed for the detection of DNA MTase based on methylation-responsive exonuclease III-assisted signal amplification. Upon the action of MTase/endonuclease on hairpin probe 1 (HP 1) containing the methylation-responsive sequence, single-stranded DNA segments are generated to hybridize with methylene blue (MB)-labeled hairpin probe 2 (HP 2). Then the digestion of HP 2 from the blunt 3′ terminus by exonuclease III is activated, resulting in the release of MB-labeled mononucleotides and the complementary DNA segment which could hybridize with another HP 2 to initiate the signal amplification process. The MB-labeled mononucleotide, due to its less negative charge and smaller size, diffuses easily to the negatively charged indium tin oxide (ITO) electrode, generating an amplified electrochemical signal. The detection limit of the proposed assay was estimated to be 0.04 U/mL, which was better than or comparable to that of the biosensors previously reported. To the best of our knowledge, it is the first time to adopt exonuclease III-assisted signal amplification for homogeneous electrochemical assay of MTase activity, and this strategy exhibits the advantages of high sensitivity as well as simplicity. Since this assay is carried out in a homogeneous solution phase instead of on an electrode/solution interface, sophisticated probe immobilization processes could be avoided. The as-proposed strategy exhibits promising potential for MTase functional studies and related researches.

1. Introduction

DNA methylation is a critical and common gene protection approach existing in both prokaryotes and eukaryotes. It is an important epigenetic event that regulates cell functions in many biological processes involved in transcription, chromatin structure, cellular differentiation and embryogenesis [1–5]. Therefore, aberrant DNA methylation may alter gene expression, influence the interaction between DNA and protein, and change normal cellular functions and phenotypes, which are related to pathogenesis of cancers [4–7]. The DNA methylation process is regulated by DNA methyltransferase (MTase) enzymes, which modify nucleic acids by recognizing the short palindromic sequences specifically and catalyzing the transfer of a methyl group from the donor S-adenosylmethionine (SAM) to the target adenine or cytosine residues in DNA [8]. In recent years, the DNA MTases have attracted much attention in the studies of cancer pathology and have been regarded as a novel family of potential targets in disease diagnosis and therapy [9]. Thus, the exploitation of sensitive and selective methods to evaluate the DNA MTase activity is highly desirable in the fields of drug discovery and clinical diagnosis.

For activity screening of DNA MTase, several conventional approaches including methylation-specific polymerase chain reaction (PCR), radioisotope labeled substrates, high-performance liquid chromatography (HPLC) and capillary electrophoresis have been developed [10–12]. Unfortunately, these methods are known to be time-consuming and require sophisticated instrumentation, various laborious treatment, and especially isotope labeling. Therefore, to overcome the drawbacks of the traditional assays, tremendous efforts have been made to assay DNA MTase activity in recent years using methods such as fluorescence, chemiluminescence, and colorimetry [13–21]. For instance, Lee et al. [16] have reported a fluorescent method for DNA MTase activity

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assay based on the preferential binding of single-stranded DNA over double-stranded DNA to graphene oxide. Quite recently, Qu's group [19] employed an enzyme-responsive nanoparticle system to demonstrate a universal colorimetric assay for DNA MTase activity by using DNA-gold nanoparticle assemblies as the substrates. In addition, the complexes of DNA and cationic conjugated polymer combined with the fluorescence resonance energy transfer were used for amplified fluorescence assay of DNA MTase activity [20]. These methods have their own advantages and partly get rid of the limitations of the conventional assays.

Compared to those previously reported methods which need sophisticated optical instruments, electrochemical methods exhibit fascinating advantages since they are impressively cost-effective and miniaturizable. Therefore, many electrochemical approaches have been developed for the detection of MTase activity [22–27]. For example, Yu's group introduced an electrochemical biosensor for the monitoring of DNA MTase activity based on the methylation sensitive cleavage using terminal transferase-mediated extension [24]. Ai and co-workers developed an electrochemical biosensing method for the detection of DNA methylation using methylene blue as the electrochemical indicator [25]. Muren and Barton [27] proposed a multiplexed, signal-on electrochemical assay for methyltransferase activity on DNA-modified electrodes. However, these electrochemical strategies for the investigation of MTase activity are based on heterogeneous assays which all need the immobilization of the DNA probes on the electrode surface and other tedious processes for the construction of electrochemical biosensors. As a result, appropriate immobilization strategies and steps are needed to overcome any steric hindrance of the electrode surfaces. Thus, the development of faster and simpler immobilization-free electrochemical approaches to assay MTase activity is still desirable. Lately, various detection platforms based on immobilization-free electrochemical strategies have been developed to detect different analytes including DNA, metal ions and small biological molecules [28–33]. Among them, many approaches take advantage of the fascinating characteristics of exonuclease (Exo) III, which is a sequence-independent nuclease and has a high exodeoxyribonuclease activity on duplex DNA with blunt ends in the direction from 3′ to 5′ terminus but limited activity on single-stranded DNA or duplex DNA with a protruding 3′ end [34]. Very recently, Wei et al. [35] reported a homogeneous electrochemical strategy for DNA methylation detection and inhibitor screening, however, since no signal amplification was adopted in this method, there is still room for further improvement of the sensitivity.

In this paper, we present a novel versatile methylation-responsive Exo III-assisted signal amplification strategy for homogeneous electrochemical assay of MTase activity. To the best of our knowledge, it is the first time to combine the homogeneous electrochemical strategy and the methylation-responsive Exo III-assisted signal amplification for the sensitive detection of MTase activity. The as-proposed strategy employs a DNA containing the methylation-responsive sequence, a methylene blue-labeled DNA and a negatively charged indium tin oxide (ITO) electrode as the working electrode. This approach relies on the specific recognition of MTase and the specific methylation-sensitive restriction endonuclease which would subsequently cleave the methylated DNA. The methylation-induced duplex DNA scission and the release of oligonucleotides could be used as a switch to activate Exo III, resulting in the Exo III-assisted signal amplification and the generation of measurable electrochemical signals. This signal-amplified method for MTase activity detection has the advantages of high sensitivity as well as simple operation, since immobilization-free electrochemical detection strategy is adopted. Accordingly, the electrochemical signal change is used to effectively assay the MTase activity and the as-proposed strategy would be a very promising candidate for MTase functional studies in the future.

2. Experimental

2.1. Reagents and materials

DNA adenine methylation (Dam) methyltransferase (MTase), DpnI endonuclease, S-adenosylmethionine (SAM) and exonuclease (Exo) III were purchased from New England Biolabs (Beijing, China) and used without further purification. Tris(hydroxymethyl)aminomethane (Tris), hydrochloric acid (HCl), NaCl, EDTA and $MgCl_2$ were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The reagents were of analytical grade and used without further purification or treatment. Double distilled water (DDW) was used throughout the experiments. All oligonucleotides used in this work were synthesized and purified by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China), and their sequences were as follows: hairpin probe 1 (HP 1), 5′-CGCTTGATCGAAC-ACCAGGTAGATACGTACGAGTGCGATCAAGCGGATTTGC-3′; hairpin probe 2 (HP 2), 5′-AGGTAGACGTACGTATCTACCTGGTGTTC-methylene blue-3′. HP 1 and HP 2 were used as provided and diluted in 10 mM Tris–HCl buffer solution (pH 7.5, containing 50 mM NaCl, 10 mM $MgCl_2$ and 1 mM EDTA) to give the stock solutions of 10 μM. Each oligonucleotide was heated to 95 °C and maintained at this temperature for 10 min, and slowly cooled down to room temperature before use.

2.2. Electrode pretreatment and electrochemical measurement

Differential pulse voltammetric (DPV) measurements were performed using a CHI 660E electrochemical analyzer (Shanghai, China). A three-electrode system was employed, with an ITO electrode with a surface area of 0.5 cm^2 as the working electrode, Ag/AgCl as the reference electrode, and a platinum wire as the auxiliary electrode. Before each electrochemical measurement, the ITO electrode was sequentially sonicated in an Alconox solution (8 g of Alconox per liter of water) for 15 min, propan-2-ol for 15 min, acetone for 15 min, and water for 15 min. Then, the ITO electrode was immersed into 1 mM NaOH solution for 5 h at room temperature and sonicated in water for 15 min. After these cleaning procedures, a negatively charged working electrode surface was obtained.

2.3. Exonuclease III-assisted assay of Dam MTase activity

The methylation experimentation was performed in 30 μL of methylase buffer (10 mM Tris–HCl, 50 mM NaCl, 10 mM $MgCl_2$, pH 7.5) containing 1 μM HP 1, 80 μM SAM, 80 units of DpnI, and a varying amount of Dam MTase at 37 °C for 2 h. After the methylation reaction, 10 μL of 10 μM HP 2 and 10 μL of 10 U/μL Exo III were added to bring the total volume to 50 μL and the resulting solution was kept at 37 °C for a certain period of time before the electrochemical measurements.

3. Results and discussion

3.1. Principle of the methylation-responsive sensing system

As a proof-of-concept, the DNA adenine methylation (Dam) MTase which catalyzes the transfer of a methyl group from SAM to the N6 position of the adenine residues in the symmetric tetranucleotide 5′-G-A-T-C-3′, and the DpnI endonuclease which can specifically recognize and cleave the fully methylated site of 5′-G-mA-T-C-3′ were chosen as the model MTase and endonuclease for