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Short communication

An electrochemical one-step system for assaying methyltransferase activity based on transport of a quantum dot signaling tracer

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ABSTRACT

A one-step, electrochemical method for assaying methyltransferase (MTase) activity, based on the convective transport of a quantum dot (QD) signaling tracer, has been developed. The assay chip used in this system was prepared by modifying a gold matrix with CdSe/ZnS QD-tagged dsDNA, which contains a specific methylation site (5′–GATC-3′) recognized by MTase. Treatment of the chip with DNA adenine methylation (Dam) MTase, generates a methylated sequence (5′-GAmTC-3′) within the dsDNA. The methylated dsDNA is then subjected to a cleavage reaction, induced by DpnI, which leads to release from the gold matrix of a DNA fragment tethered to a QD. Detection of the released QD, using square wave anodic stripping voltammetry (SWASV) on a glassy carbon (GC) electrode, enables the reliable quantitation of the methylated DNA. Because it is accomplished in a simple and convenient one step and does not require any complicated secondary or tedious washing steps, the new assay method holds great promise for epigenetic analysis in facility-limited environments or point-of-care testing (POCT) applications.

1. Introduction

DNA methylation has received increased attention recently because it is a significant and critical epigenetic process that regulates cell function by changing gene expression (Choy et al., 2010; Heithoff et al., 1999; Shames et al., 2007). The methylation reaction occurring in both prokaryotes and eukaryotes involves DNA methyltransferases (MTase) catalyzed covalent addition of a methyl group to positions in cytosine or adenine groups in DNA. DNA MTases recognize methylation-specific sequences in DNA and then promotes methylation utilizing S-adenosyl-L-methionine (SAM) as the methyl donor (Jeltsch, 2002; Robertson and Wolffe, 2000). Recent studies have shown that aberrant DNA methylation might serve as a new generation of cancer biomarkers because it is critically involved in tumor growth. Consequently, MTases are under intense investigation as potential targets for anticancer therapy. Because of this interest, a great demand exists for reliable methods to assess DNA methylation (Brueckner and Lyko, 2004; Egger et al., 2004; Jones and Baylin, 2002).

Traditional techniques to detect DNA methylation or assess the activity of DNA MTases include the use of ^{3}H NMR, blotting, high performance liquid chromatography (HPLC), mass spectrometry (MS), and gel electrophoresis. Unfortunately, all of these methods require cumbersome procedures, such as radioactive labeling or conversion of un-methylated cytosine to uracil by using sodium bisulfate (Bergerat et al., 1991; Boye et al., 1992; Friso et al., 2002; Herman et al., 1996; Lo et al., 1999). As a result, they are not only time-consuming and labor-intensive but also suffer from crucial limitations that diminish the overall reliability of the assay. In addition, their drawbacks lead to high costs and operational inconveniences that significantly limit their practical use in relevant assay fields.

To overcome the limitations of the methods described above, various strategies have been described for the detection of DNA methylation or the assessment of the activity of DNA MTase. These include colorimetric assays utilizing methylation-responsive DNA-zyme or gold nanoparticles (AuNPs), and fluorescent methods employing dye-tagged hairpin DNA probes (Li et al., 2007; Li et al., 2010; Liu et al., 2010). Among the most promising analytical methods are those that are based on electrochemical responses, owing to several advantageous characteristics that include simplicity, portability and cost effectiveness (Bakker, 2004; Drummond et al., 2003; Won et al., 2011a, 2011b). However, the electrochemical methods reported to date also require utilization of laborious multi-step procedures that include complicated secondary and tedious washing steps (Liu et al., 2011). Therefore, a great need exists for the development of more convenient, one-step electrochemical methods to assess MTase activity in a reliable manner. To meet this need, we carried out a recent investigation targeted at the development of a novel electrochemical, one-step method for assaying MTase activity.

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2. Material and methods

2.1. Chemicals and reagents

DNA adenine methylation (Dam) MTase, EcoRI MTase, DpnI restriction endonuclease, and S-adenosyl-L-methiolnine (SAM) were purchased from New England Biolabs (NEB) Inc. All anticancer drugs (inhibitors), Tris(hydroxymethyl)aminomethane, hydrochloride, sodium chloride, magnesium chloride, and other salts were obtained from Sigma-Aldrich and used without further purification. Streptavidin-coated CdSe/ZnS QD was purchased from Invitrogen Corporation. Ultrapure water was obtained using a Millipore filtration system. All synthesized oligonucleotides were obtained from IDT (Integrated DNA Technologies) and purified by using HPLC, and their identities were confirmed by using MALDI-TOF. The sequences of DNA oligonucleotides used in this work are as follows:

Capture probe: 5′–GAG AGG AAT AGT GAT CAT TGT TAT TAG GAA-$(A)_{10}$-thiol-3′
Biotin labeled probe: 5′- CCT AAT AAC AAT GAT CAC TAT TCC TCT CAA-$(A)_{10}$-biotin-3′

2.2. Fabrication of the assay chip

A gold matrix (Shin et al., 2011b) was fabricated by coating 20 nm titanium on a silicon wafer (1 0 0) followed by 200 nm gold (99.999%) thin layer formation by using an e-beam evaporator. The gold matrix was cleaned with piranha solution (H_2SO_4: H_2O_2=4:1) for 20 min and then thoroughly washed with phosphate buffered saline (PBS) and treated with 100 μL of an aqueous solution containing 100 nM thiolated capture probe for 2 h. The amount of DNA immobilized on the electrode surface was determined by evaluating the absorbance difference of capture probe solutions before and after immobilization process using a NanoDrop® ND-1000 UV–vis Spectrophotometer (Shin et al., 2011a). After washing with PBS, the DNA-modified matrix was treated with 100 μL of 1 mM mercaptohexanol solution for 15 min in order to block parts of the gold surface that are not covered by the capture probe. Finally, the gold matrix surface was washed with PBS and water. A biotin-labeled complementary strand was allowed to hybridize with the capture probe on the gold matrix surface in a humid chamber at 25 °C for 1 h and the resulting modified gold matrix was washed with PBS. Subsequently, 20 nM of streptavidin-coated CdSe/ZnS QD in 100 μL PBS was added and allowed to react with the biotin moiety of the DNA immobilized on the gold matrix in a humid chamber at 25 °C for 1 h. The fabricated assay chip was washed with PBS and MTase buffer (pH 7.5, 50 mM NaCl, 10 mM Tris–HCl, and 10 mM $MgCl_2$) and used immediately.

2.3. Assay procedures for Dam MTase activity or inhibitory activity against Dam MTase

Aliquots of 100 μL of MTase buffer, containing various amounts of Dam MTase from 1.0 to 128.0 U/mL, 80 μM of SAM, and 20 units of DpnI, were introduced to the assay chip and allowed to react at 37 °C for 2 h. The solution was then collected from gold matrix and treated with strong acid (2.3 M HNO_3) to dissolve the QDs for 30 min. Square wave anodic stripping voltammetry (SWASV) was performed to detect the dissolved QDs in the presence of 0.25 mM mercury ion, required to form mercury film electrode (MFE). A conventional three-electrode cell including a glassy carbon (GC) working electrode (3-mm in diameter, CH Instruments), platinum counter electrode, and Ag/AgCl reference electrode was used for electrochemical measurements. The distances between three electrodes and gold matrix surface were fixed at 1 mm. Prior to use, the GC working electrode was polished sequentially with slurries of 0.3 and 0.05 μm alumina to create a mirror finish, and sonicated with double distilled water for about 10 s, which was then rinsed thoroughly with double distilled water and dried under ambient temperature. Square wave anodic stripping voltammetry (SWASV) was performed using an electrochemical analyzer (GAMRY, Reference 600, Warminster, PA) connected to a computer for data analysis. SWASV was measured under the following conditions: initial potential=−1.4 V, final potential=−0.1 V, pulse size potential=25 mV, step size=0.05 V, frequency=25 Hz, accumulation time=120 s, and equilibrium time=10 s.

To assess the selectivity of our method, we repeated the same assay procedure by using EcoRI MTase, which methylates a different site from Dam MTase. The detailed procedure for the assay is described in the Supporting Information.

In the assay system used to determine inhibitory activities of anticancer drugs against Dam MTase, the same procedure used for assessing Dam MTase activity was employed. In this case, the MTase buffer solution contained 128 U/mL of Dam MTase and 1 μM of additional inhibitory drugs. In particular, the inhibition effect of 5-fluorouracil was quantitatively analyzed as a model case.

3. Results and discussion

3.1. The overall procedure

The method is based on the convective transport of a quantum dot (QD) signaling tracer, which is released by enzymatic cleavage reaction of methylated DNA. For this purpose, a chip with a 5′-thiol-modified capture probe, containing the specific recognition sequence (5′–GATC-3′) of Dam MTase, was immobilized on the surface of a gold matrix (Fig. 1A). Complementary DNA, labeled with biotin at the 5′-end is then hybridized to the immobilized capture probe. Finally, a streptavidin-coated CdSe/ZnS QD is applied in order to bind with the biotin moiety of the immobilized dsDNA, thus creating a gold matrix modified with the DNA–QD complex.

To determine its MTase activity, a sample together with DpnI is applied to the QD-tagged dsDNA modified gold matrix. Dam MTase present in the sample then catalyzes the formation of the methylated sequence (5′-GAmTC-3′) in the immobilized dsDNA. The methylated dsDNA is cleaved by DpnI to produce a DNA fragment tethered to the QD, which is then released from the matrix. Because it is free to move to the GC electrode by convective transport, the QD can promote generation of an electrochemical signal through SWASV on an additional GC working electrode positioned 1 mm from the gold matrix. On the other hand, if no Dam MTase activity exists in the sample and, therefore, no methylated dsDNA site is formed on the assay chip, the QD remains attached to the gold matrix even after the treatment with DpnI. In this event, no electrochemical signal is produced (Fig. 1B).

3.2. Optimization of the amount of capture probe

The strategy used to design the novel electrochemical system for assessing MTase activity relies on the release and convective transport of QDs tethered to the capture probe DNA once released from the gold matrix. Consequently, the electrochemical signal generated by this process should be directly proportional to the amount of the immobilized capture probe carrying the QDs. To determine the amount of QD-tagged capture probe that is required on the matrix to give optimal results, assays for MTase activity were carried out using several different assay chips, prepared by employing various concentrations (10 nM–1 μM) of the capture probe solutions. The results of this study (Fig. S1 in Supporting