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Commercial glucometer as signal transducer for simple evaluation of DNA methyltransferase activity and inhibitors screening

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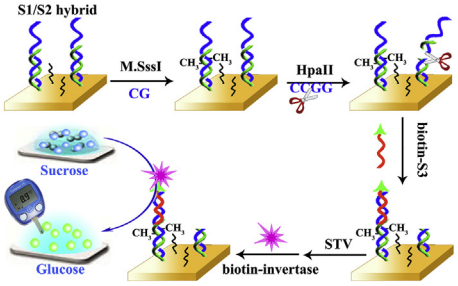
HIGHLIGHTS

- A glucometer is used in sensitive DNA MTase detection.
- The operation process is simple.
- The method shows the ability for real sample analysis.

GRAPHICAL ABSTRACT



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ABSTRACT

DNA methyltransferase (MTase) plays an important role in many biological processes and has been recognized as a predictive cancer biomarker far before other signs of malignancy and a therapeutic target in cancer treatment. Thus simple and sensitive determination of DNA MTase activity is urgently required. The commercially available glucometer is considered as the most successful point-of-care (POC) sensor up to date, and researchers extend its application in monitoring different types of targets rather than only glucose. Here, we developed a simple strategy for the sensitive detection of the DNA MTase (using M.SssI as an example) activity by using a glucometer as the signal transducer. A S1/S2 hybrid probe was designed including a specific recognition sequence for both DNA MTase and restriction endonuclease, and a complementary sequence for biotin-S3. Firstly, the S1/S2 hybrid probe was self-assembled on the gold electrode and methylated by M.SssI MTase to form the methylated dsDNA. Then, *HpaII* endonuclease specifically cleaved the residue of the unmethylated dsDNA. Subsequently, biotin-S3 hybridized with the overhang sequence of the methylated dsDNA. Finally, the biotin tag was successively combined with streptavidin (STV) and biotin-invertase. The invertase efficiently catalyzed the hydrolysis of sucrose to generate abundant glucose, which led to an amplified response of glucometer. This strategy could detect DNA MTase activity as low as 0.3 U mL^{-1} with good selectivity against other two cytosine MTases (*Hae*III MTase and *Alu*I MTase), and be successfully applied for screening the DNA MTase inhibitors (5-azacytidine and 5-aza-2′-deoxycytidine), implying our proposed method holds great promising application in early cancer diagnosis and therapeutics.

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1. Introduction

In recent years, DNA methylation has attracted considerable attention due to its critical role in the regulation of gene expression, cellular differentiation, and pathogenesis of various cancerous human diseases [1–5]. The DNA methylation level is closely associated with the activity of DNA methyltransferase (MTase) which can catalyze the transfer of a methyl group from the donor S-adenosyl-Lmethionine (SAM) to the 5′-position of cytosine in the CpG dinucleotides [6]. Usually, aberrant DNA MTase activity results in the aberrant DNA methylation [7], which affects the human biological processes and causes different types of diseases even including cancer [8]. Thus, DNA MTases have been served as predictive biomarkers and potential therapeutic targets in the diagnosis and prognostics of some types of diseases. In addition, abnormalities in DNA MTase activity are usually present at very low levels during the early stages of cancer development when other signs of malignancy has not appeared, implying the ability to be used for early cancer diagnosis [9,10]. Therefore, sensitive analysis of the DNA MTase activity and its inhibitors screening represent a valuable strategy to both clinical diagnostics and therapeutics.

Nowadays, there are a variety of methods being developed for the detection of DNA MTase activity. Among these methods, radioactive labeling is the current standard for DNA MTase activity analysis [11], which suffers from the radioactive hazard. To overcome this disadvantage, alternative methods including high-performance liquid chromatography (HPLC) [12], fluorescent [13,14], chemiluminescent [15], colorimetric [16,17] and electrochemical methods [7,18,19] have been developed for DNA MTase activity assay. Although well established, most of these methods are not only time consuming, but also laborious with the involvement of expensive equipment and professional operators. This means that they are only reliable in laboratory settings, which limits their wide utilizations in common practice especially the point-of-care (POC) applications. Therefore, the DNA MTase activity assay method with further development and cost reductions is urgently required to achieve POC applications in hospitals, the doctor's office or perhaps even at home. Considering the great potential of POC technology for better screening of at-risk patients, surveillance of disease recurrence and monitoring of treatment [20], the translation of clinical apparatus to at-home use remains a major challenge.

Glucometer has become one of the dominating commercially available POC diagnosis devices since its first discovery by using glucose oxidase for glucose monitoring in blood plasma in 1962 [21]. After decades of development, glucometer is selected as the most successful POC device due to its excellent advantages in terms of portable pocket size, low cost, reliable quantitative results and ease of use over other detection devices. Moreover, the recent integration of glucometer with cell phone may lead to an even rising number of users [22]. However, the glucometer responds only to glucose, which limits its further applications. To meet the needs of being applied to analyze other biomolecules beyond glucose such as protein and DNA biomarkers, an indirect method was fabricated by using the invertase enzyme as label and a glucometer as signal transducer [23–25]. The invertase catalyzes the hydrolysis of sucrose to glucose which is monitored by the glucometer. Such method brings about a revolutionary change in the POC testing of the diagnostic field.

DNA MTase is an even more significant potential biomarker than antigen protein or DNA in early diagnosis of cancer, while there were few reports about the glucometer-based detection scheme for the DNA MTase analysis. Therefore, we develop a simple strategy for the sensitive detection of the DNA MTase (using M.SssI as an example) activity by using a glucometer as the signal transducer. The invertase enzymes were introduced as labels to achieve the catalytic hydrolysis of sucrose to generate glucose monitored by a glucometer. The glucometer reading is proportional to the amount of the DNA MTase. Attributing to the high efficiency of enzyme catalysis, trace amount of target analyte indirectly causes the generation of abundant glucose (fall in the dynamic range of the glucometer: 0.6 mM–33.3 mM) to amplify the signal response of the glucometer for quantitative analysis. Moreover, the specific binding of streptavidin (STV) and biotin is employed, which not only benefits the selectivity of this sensor but also facilitates the attachment of invertase labels onto the sensing surface and thus simplify the operation process.

2. Experimental

2.1. Materials and reagents

Sulfo-NHS-LC-biotinylation kit was obtained from Pierce Biotechnology (Rockford, IL, USA). M.SssI CpG methyltransferase (M.SssI MTase) supplied with 10 × NEBuffer 2 and sadenosyl-L-methiolnine (SAM), restriction endonuclease *HpaII* supplied with 10 × CutSmart buffer, *Hae*III MTase and *Alu*I MTase were from New England Biolabs (Ipswich, MA, USA). Tris (2-carboxyethyl)phosphine hydrochloride (TCEP), 6-mercaptohexanol (MCH), streptavidin (STV), 5-Azacytidine (5-Aza), 5-aza-2′-deoxycytidine (5-Aza-dC), invertase from baker's yeast, human serum samples and sucrose were purchased from Sigma-Aldrich. Tris (hydroxymethyl) aminomethane (Tris) and ethylenediaminetetraacetic acid (EDTA) were purchased from Solarbio (Beijing, China). All other chemicals (analytical grade) were obtained from Kelong Chemical Inc. (Chengdu, China) and used as received without further purification.

0.1 M sodium phosphate buffered saline containing 0.15 M NaCl (PBS, pH 7.2) supplied in Sulfo-NHS-LC-biotinylation kit was used for coupling the biotin with STV. The hybridization buffer (HB) was prepared in our laboratory and made of 10 mM Tris-HCl, 1 mM EDTA, 1 M NaCl (pH 7.4). All synthetic oligonucleotides were ordered from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China), and the sequences were listed below:

S1: 5' - SH-$(CH_2)_6$-CAG T**CC GG**A GGT GAA CCT TAG ATA GAC CAA TTA-3′
S2: 5' - CAC CT**C CGG** ACT G - 3′
S3: 5' - biotin - TAA TTG GTC TAT CTA AGG TT - 3′

All reagents were analytical grade and solutions were prepared using ultrapure water (specific resistance of 18 MΩ cm).

2.2. Apparatus

Cyclic voltammetry (CV) was performed on a CHI 660C electrochemical workstation (Chenhua Instrument Company of Shanghai, China). A conventional three-electrode configuration was used with the modified gold working electrode (AuE, 3 mm in diameter) as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. The glucometer (Contour™TS) and test strips were from Bayer Healthcare LLC (Mishawaka, IN).

2.3. Preparation of the biotin-invertase

Previous researches have indicated that the reaction with amines of invertase is insusceptible to the enzyme natural activity [23,26]. A sulfo-NHS-LC-biotinylation kit was used for the conjugation of NHS esters of biotin reagents and amines of invertase via a