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Magnetic bead-liposome hybrids enable sensitive and portable detection of DNA methyltransferase activity using personal glucose meter

Youna Zhang [a], Qingwang Xue [a,*], Jifeng Liu [b,*], Huaisheng Wang [a,*]

[a] Department of Chemistry, Liaocheng University, Liaocheng, Shandong 252059, China
[b] Key Laboratory of Food Nutrition and Safety, Ministry of Education of China, Tianjin University of Science and Technology, Tianjin 300457, China

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ABSTRACT

DNA methyltransferase (MTase) plays a critical role in maintaining genome methylation patterns, which has a close relationship to cancer and bacterial diseases. This encouraged the need to develop highly sensitive, simple, and robust assays for DNA MTase detection and inhibitor screening. Herein, a simple, sensitive, and specific DNA MTase activity assay was developed based on magnetic beads-liposome hybrids combined with personal glucose meter (PGM) for quantitative detection of DNA MTase and inhibitor screening. First, a magnetic beads-liposome hybrid probe is designed by the hybridization of p_1DNA-functionalized magnetic bead with p_2DNA-functionalized glucoamylase-encapsulated liposome (GEL). It integrates target recognition, magnetic separation and signal amplification within one multifunctional design. Then, in the presence of Dam MTase, the hybrids probe was methylated, and cleaved by methylation-sensitive restriction endonuclease Dpn I, making liposome separated from magnetic bead by magnetic separation. Finally, the separated liposome was decomposed, liberating the encapsulated glucoamylase to catalyze the hydrolysis of the signal substrate amylose with multiple turnovers, producing a large amount of glucose for quantitative readout by the PGM. In the proposed assay, the magnetic beads-liposome hybrids offered excellent sensitivity due to primary amplification via releasing numerous glucoamylase from a liposome followed by a secondary enzymatic amplification. The use of portable quantitative device PGM bypasses the requirement of complicated instruments and sophisticated operations, making the method simple and feasible for on-site detection. Moreover, the proposed assay was successfully applied in complex biological matrix and screen suitable inhibitor drugs for DAM for disease(s) treatment. The results reveal that the approach provides a simple, sensitive, and robust platform for DNA MTases detection and screening potential drugs in medical research and early clinical diagnostics.

1. Introduction

DNA methyltransferase (MTase) is responsible for maintaining methylation patterns in the genome, which are vital to the regulation of gene expression and maintenance of genomic stability and integrity (Cheng and Roberts, 2001; Choy et al., 2010; Ferguson-Smith and Surani, 2001). Aberrant expression of DNA MTase may lead to aberrant DNA methylation partterns, which can silence the tumor suppressor genes and promote cancerous transformations (Chen et al., 1998; Muren and Barton, 2013). Recent research has demonstrated that aberrant DNA MTase activity was related to pathogenesis of cancer, such relationship provides a potential target in disease diagnosis and therapy. (Mutze et al., 2011) Moreover, aberrant activity of MTase usually occurs before other signs of malignancy, and thus exhibits potential use for early cancer diagnosis (Baylin and Herman, 2000; Das and Singal, 2004). Thus, simple and sensitive monitoring of MTase activity and inhibitor screening for MTase activity inhibition are of great importance for both clinical diagnostics and drug development.

Traditionally, radioactive labeling strategy (Som and Friedman, 1991), various PCR (polymerase chain reaction)-based techniques (Lyko et al., 2000), high performance liquid chromatography (Friso et al., 2002), gel electrophoresis (Torres et al., 2011), and immune-based assays (Boye et al., 1992; Wang et al., 2012) are well established for MTase activity analysis. Additionally, some of new methods have been developed for MTase activity assay, which include fluorescence assays (Ouyang et al., 2012; Tian et al., 2012),

* Corresponding authors.
E-mail addresses: xueqingwang1983@163.com (Q. Xue), liujifeng111@gmail.com (J. Liu), hswang@lcu.edu.cn (H. Wang).

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electrochemical assays (Kato et al., 2008; Li et al., 2012; Muren and Barton, 2013), colorimetric assays (Liu et al., 2010), bioluminescence assays (Hendricks et al., 2004; Jiang et al., 2010), chemiluminescence assays (Zeng et al., 2013), and electrogenerated chemiluminescence assays (He et al., 2011). Although these methods are well-established, most of them are laborious with the involvement of expensive equipment and they are not widely available to the public due to bulky and expensive signal instruments. Consequently, the design of convenient and inexpensive approaches for the sensitive detection of MTase activity with rapid, easy manipulation is in ever-increasing demand.

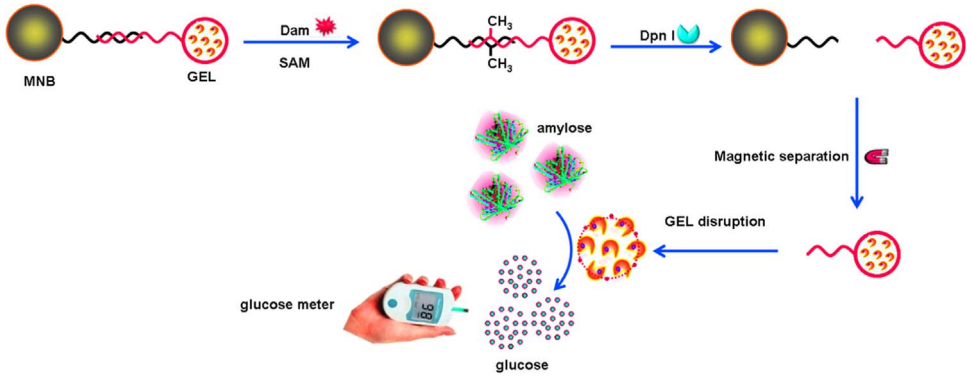
Typically, DNA MTase assay is performed using the DNA molecular probe-based assay system, in which DNA MTase can specifically act on the DNA recognition sites for DNA methylation and then the methylation event is detected via methylation-sensitive restriction enzyme cleavage. To improve the sensitivity, signal amplification strategies are often combined in the fabrication of MTase activity assay (Xue et al., 2015). Generally, signal amplification includes the use of nanomaterial as amplifying labels (He et al., 2011; Li et al., 2012; Wang et al., 2012) and oligonucleotides isothermal amplification techniques (Bi et al., 2013; Li et al., 2012; Xing et al., 2014; Zeng et al., 2013; Zhao et al., 2013). A variety of DNA MTase activity assays relying on signal amplification strategies have been developed. Although a high sensitivity can be achieved, these methods usually involve multiple assay steps and require the addition of many exogenous reagents (Liu et al., 2013). Especially, oligonucleotides isothermal amplification via nuclease is prone to yielding false positive results, which may have effect on the detection specificity and sensitivity (Zhao et al., 2016). Thus, it is highly required to develop a sensitive, specific, and simple approach for determination of DNA methylation and assay of MTases activity.

Herein, we develop a simple, sensitive and specific DNA MTase activity assay based on magnetic beads-liposome hybrids combined with personal glucose meter for portable and quantitative detection of DNA MTase. The designed strategy is depicted in Scheme 1. A magnetic beads-liposome hybrids probe is designed by the hybridization of p_1DNA-functionalized magnetic bead and p_2DNA-functionalized glucoamylase-encapsulated liposome (GEL). It integrates target recognition, magnetic separation, and signal amplification within one multifunctional design. In the presence of Dam MTase, the magnetic beads-liposome hybrids probe was methylated and then was cleaved by the methylation-sensitive restriction endonuclease Dpn I. The cleavage makes GEL apart from the magnetic bead and be separated by magnetic separation. The separated GEL can be decomposed, liberating the encapsulated glucoamylase to catalyze the hydrolysis of the signal substrate amylose with multiple turnovers, producing a large amount of glucose for quantitative readout by using an off-the-shelf PGM. In the proposed assay, an ultrahigh sensitivity was provided by primary amplification via releasing numerous glucoamylase from liposomes followed by a secondary enzymatic amplification. The methylation-sensitive restriction enzyme used here guarantees the high specificity. The use of portable quantitative device PGM bypasses the requirement of complicated instruments and sophisticated operations, making the method simple and feasible for on-site detection. Moreover, the proposed sensing assay was also successfully applied in complex biological matrix and screen suitable inhibitor drugs, indicating that the approach will become a reliable and sensitive DNA MTases quantification method in medical research and early clinical diagnostics.

2. Experimental section

2.1. Chemicals and materials

All oligonucleotide sequences were synthesized and purified by HPLC at Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences of two oligonucleotides are as follows: the biotinylated DNA probe 1 (p_1DNA), 5′-ACG A*GA TC*A AGG AGA-A_{20}-biotin-3′; the amino-terminated DNA probe 2 (p_2DNA), 5′-TCT CCT T*GA TC*T CGT-A_{20}-NH_2-3′. 1,2-distearoyl-*sn*-glycero −3-phosphocholine (DSPC), cholesterol, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol) −2000] (ammonium salt) (DSPE-PEG-COOH) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL,). Streptavidin-modified magnetic beads (MNBs) (830 nm diameter, aqueous suspension containing 100 mM borate, 0.1% BSA, 0.05% Tween-20, and 10 mM EDTA at a concentration of 1.527×10^{10} beads mL^{-1}) were obtained from Bangs Laboratories Inc. (Fishers, IN). Methyltransferase of DNA adenine methylation (Dam), methyltransferases of DNA cytosine methylation (M.sssI), HhaI methyltransferase, endonuclease of Dpn I and S-adenosylmethionine (SAM) were obtained from New England Biolabs Inc. Gentamycin and benzylpenicillin were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). Glucoamylase and amylose were purchased from Sigma-Aldrich. Other chemicals (analytical grade) were obtained from standard reagent suppliers. Water ($\geq 18.2\ M\Omega$) was used and sterilized throughout the experiments.



Scheme 1. Schematic illustration of DNA MTase activity assay based on magnetic beads-liposome hybrids-constructed cascade amplification and personal glucose meter. First, a magnetic beads-liposome hybrid is prepared by the hybridization of DNA-functionalized magnetic bead (MNB) and DNA-functionalized glucoamylase-encapsulated liposome (GEL). Then, in the presence of Dam MTase, the hybrids probe was methylated with the help of SAM and cleaved by methylation-sensitive restriction endonuclease Dpn I, making liposome separated from magnetic bead by magnetic separation. Finally, the separated liposome was decomposed, liberating the glucoamylase encapsulated, which catalyzed the hydrolysis of the signal substrate amylose with multiple turnovers, producing a large amount of glucose for quantitative readout by the PGM.