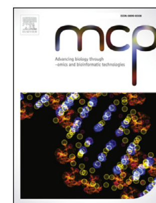
Molecular and Cellular Probes 30 (2016) 118–121

Contents lists available at ScienceDirect

Molecular and Cellular Probes

journal homepage: www.elsevier.com/locate/ymcpr



Short communication

Label-free monitoring of DNA methyltransferase activity based on terminal deoxynucleotidyl transferase using a thioflavin T probe

Changbei Ma [a,*], Haisheng Liu [a], Wei Li [b], Hanchun Chen [a], Shunxin Jin [a], Jing Wang [a], Jun Wang [a,**]

[a] State Key Laboratory of Medical Genetics & School of Life Sciences, Central South University, Changsha 410013, China
[b] School of Chemistry and Biological Engineering, Changsha University of Science and Technology, Changsha, 410004, China

ARTICLE INFO

Article history:
Received 1 December 2015
Received in revised form
9 January 2016
Accepted 2 February 2016
Available online 5 February 2016

Keywords:
Label-free
DNA methyltransferase
Thioflavin T
Terminal deoxynucleotidyl transferase

ABSTRACT

We have developed a new methodology for fluorescence turn-on detection of DNA methyltransferase (MTase) activity based on terminal deoxynucleotidyl transferase (TdT) using a thioflavin T probe. This method is highly selective and sensitive. The fluorescence intensity was direct proportion to Dam MTase concentration in the range from 0.1 to 8.0 U/mL with a detection limit of 0.1 U/mL. And because no labeling with a fluorophore–quencher pair was required, it is simple and low cost. We envision that our novel fluorescent detection method for Dam MTase activity could be applied as a useful tool in biomedical research.

© 2016 Elsevier Ltd. All rights reserved.

DNA methylation is an important epigenetic modification both in eukaryotes and prokaryotes, and plays crucial roles in the regulation of gene expression, genomic stability and cellular differentiation [1]. A growing number of human diseases have been found to be associated with aberrant DNA methylation. DNA methylation is proceed by DNA methyltransferase (MTase) which can recognize specific sequences and transfer a methyl group from the donor S-adenosylmethionine (SAM) to target cytosine or adenine [2], and DNA MTase may become a potential target for anticancer therapy and drug screening. Therefore, the monitoring of DNA MTase activity is crucial to clinical diagnostics, disease therapy and biomedical research.

Many traditional assay methods, such as gel electrophoresis, radioactive labeling, high performance liquid chromatography (HPLC), and enzyme-linked immunosorbent assay (ELISA), have been developed for the detection of DNA MTase activity [3,4]. However, these methods have some drawbacks, including laborious and time-consuming operation, dangerous radio-labeled substrates, expensive equipment, or costly antibodies. To overcome the shortcomings of the traditional methods, alternative methods have been developed, such as fluorescence assays [5,6], colorimetric detection [7], bioluminescence assay [8], photoelectrochemical methods [9], and electrochemical methods [10–12]. Fluorometric methods have drawn considerable attention because the assay is convenient and the sensitivity is usually high. For example, previously, the molecular beacon-based fluorescence method has been developed for DNA MTase activity [13]. Recently, Roach et al. reported a real-time assay for CpG-specific cytosine-C5 methyltransferase activity using a hemimethylated molecular break light oligonucleotide probe [14]. However, design and synthesis of double labeled molecular beacon probes are relatively expensive and complex which may limit its practical use. Very recently, Zhao and Gao groups realized the detection of DNA MTase activity by using graphene oxide or MoS2 nanosheets as a super quencher [15,16]. However, synthesis of graphene oxide or MoS2 nanosheets is time-consuming and the quenching ability is unstable. Therefore, the development of a label-free, sensitive and cost-effective method for monitoring of DNA MTase activity is of considerable interest.

Recently, some small molecules have been demonstrated to selectively bind to G-quadruplexes resulting in a significant enhancement of fluorescence, and leading to the development of G-quadruplex based fluorescent sensors [17–19]. Among these small molecules, Thioflavin T (ThT) has proved a novel fluorogenic dye. Recently, Mohanty et al. reported that ThT can specifically bind to a

* Corresponding author.
** Corresponding author.
E-mail addresses: macb2012@csu.edu.cn (C. Ma), junwang@csu.edu.cn (J. Wang).

http://dx.doi.org/10.1016/j.mcp.2016.02.001
0890-8508/© 2016 Elsevier Ltd. All rights reserved.

DNA G-quadruplex derived from the human telomeric repeat sequence, generating a highly specific fluorescence enhancement [20]. Due to their inherent advantages such as low background fluorescence, high analyte dependence and low cost, several ThT based fluorescent biomolecules and metal ion biosensors have been reported [21–23]. For instance, Tan and co-workers reported a simple approach for specific detection of DNA, RNA and protein [24]. To the best of our knowledge, the exploration of ThT probe is still at a very early stage and has a great potential to be utilized in biochemical and biomedical applications. In this work, we proposed to develop a sensitive, simple, low cost, and label-free approach for DNA MTase assay based on terminal deoxynucleotidyl transferase (TdT) using a ThT probe.

A schematic illustration of the analysis of DNA MTase activity is shown in Scheme 1. A hairpin DNA as a recognition probe which is phosphorylated at its 3′ termini is designed. Dam MTase and DpnI restriction endonuclease are chosen as the model MTase and endonuclease, respectively. Since DNA probe contained no free 3′-OH functional groups in the absence of Dam MTase, it could not be elongated by TdT. In the presence of Dam MTase, it could methylate the recognition sequence to generate the methylation DNA (5′-G-mA-T-C-3′), acting as the substrate for DpnI. Then, DpnI cleaves the methylated probe into three short single-stranded DNA fragments containing two free 3′-OH termini. The DNA fragments contained the free 3′-OH termini could be very much elongated by TdT. The TdT-produced G-rich products can be recognized quickly by the ThT dyes [25]. Correspondingly, the fluorescence intensity of the reaction mixture at 490 nm increased greatly. According to the fluorescence signal change of the detection system, we can easily detect Dam MTase activity.

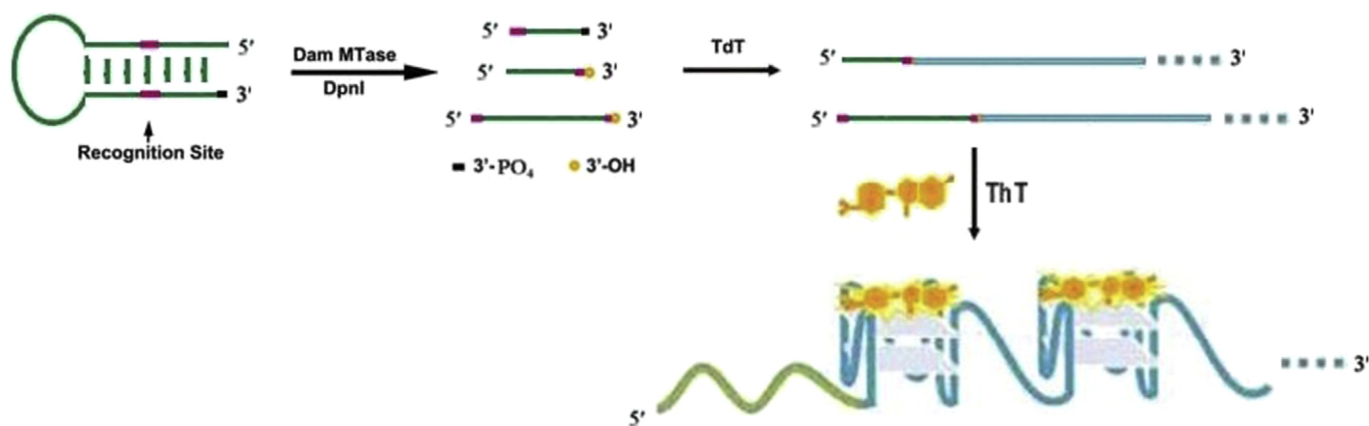
To demonstrate the feasibility of our proposed, two samples were prepared. Sample A contains DNA probe (5′-GAGGGCCTG-CAGGATCATTGGCTTTTGCCAATGATCCTGCA GGCCCTC-p-3′, the p represented the phosphate at 3′ end) and DpnI. Sample B contains DNA probe, DpnI and Dam MTase. The concentration of DNA probe, DpnI and Dam MTase was 625 nM, 640 U/mL and 12 U/mL, respectively. The buffer containing 20 mM Tris-HAc, pH 7.9, 50 mM KAc, 10 mM $Mg(Ac)_2$, and 1 mM DTT was first used in the methylation reaction. Samples solutions were incubated at 37 °C for 1 h. Then 6.4 units of TdT, 10 mM of dGTP, and reaction buffer (100 mM HEPES, pH 7.2, 8 mM $MgCl_2$, 0.1 mM DTT) were added. Sample solutions were incubated at 37 °C for 2 h. Finally, 2 μM of ThT was added, and fluorescence intensity was obtained on an F2700 (Hitachi, Japan) with excitation at 425 nm, and the emission spectra were collected from 460 to 600 nm.

Fig. 1A shows the fluorescence emission spectra of the DNA probe both in the absence (curves a) and presence (curves b) of Dam MTase. As is demonstrated in Fig. 1A, the fluorescence emission intensity was quite low in the absence of Dam MTase (curve a). This is as expected; in the absence of Dam MTase, DNA probe contained no free 3′-OH termini, it could not be elongated by TdT. As shown in curve b, in the presence of 12 U/mL Dam MTase, a significant enhancement in fluorescence intensity was observed, which confirms that the DNA methylation and cleavage process helps the enzymatic extension reaction to occur. Thus, an ultrahigh S/B ratio of 33.2 fold was obtained in fluorescence measurement, providing a sensitive fluorescence assay for Dam MTase activity.

We investigated a series of reactions using various concentrations of Dam MTase at 0, 0.1, 0.2, 0.8, 2.0, 4.0 and 8.0 U/mL respectively (Fig. 1B). The result indicated that as the Dam MTase concentration was increased, the fluorescence enhancement rate was increased accordingly. With the enzyme ranging from 0.1 to 8.0 U/mL, a good linear correlation ($r^2 = 0.9911$) between fluorescence intensity and Dam MTase concentration was observed (Fig. 1C). The assay is highly sensitive, a Dam MTase detection limit of 0.1 U/mL was obtained, which is comparable with or better than some commonly used techniques, such as colorimetric assay (with a detection limit of 6 U/mL) [26], fluorescence detection (with a detection limit of 0.4 U/mL) [27], chemiluminescence (with a detection limit of 0.52 U/mL) [28], and electrochemical method (with a detection limit of 0.1 U/mL) [11]. This assay is convenient, sensitive, and low cost, which makes it a useful approach to analyze the activity of Dam MTase.

The selectivity of the proposed Dam MTase activity assay was further investigated using M.SssI MTase and AluI MTase as the potential interference enzymes. They are also methyltransferase. AluI MTase specifically methylates the cytosine residue (C5) of the sequence 5′-AGCT-3′ within the duplex DNA, and M.SssI MTase specifically methylates the cytosine residue (C5) within the double-stranded DNA recognition sequence of 5′-CG-3′. Fig. 2A shows that 12 U/mL Dam MTase could induce a significant fluorescence increase. In contrast, M.SssI MTase and AluI MTase at the same concentration could not induce noticeable fluorescence changes. This is due to the fact that the site in the DpnI endonuclease recognition sequence (5′-GATC-3′) cannot be methylated by the M.SssI and AluI cytosine MTase. Therefore, the results clearly suggest that our assay is highly selective for Dam MTase.

The established strategy can be further adapted to study the



Scheme 1. Schematic illustration of Dam MTase activity detection. In the presence of Dam MTase and DpnI endonuclease, the DNA could be specifically methylated and then cleaved into single-stranded fragments. The DNA fragments contained newly generated 3′-OH termini, which could be elongated by TdT. The TdT-produced G-rich products can be recognized quickly by the ThT dye and resulting in an increase in fluorescence.

Download English Version:

https://daneshyari.com/en/article/2199658

Download Persian Version:

https://daneshyari.com/article/2199658

Daneshyari.com