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An electrochemical assay for DNA methylation, methyltransferase activity and inhibitor screening based on methyl binding domain protein

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ABSTRACT

DNA methylation is one of important epigenetics events, and responsible to transcription, genomic imprinting and cellular differentiation. Aberrant DNA methylation is always contacted with various diseases. Methyl binding domain (MBD) proteins can specifically bind to the methylated CpG dinucleotides. Conventional assay for DNA methylation normally need bisulfide treatment, methylated nucleotide labeling or PCR amplification. Here, we fabricated a novel electrochemical biosensor for detection of DNA methylation, assay of DNA methyltransferase (MTase) activity and screening of MTase inhibitor based on MBD protein and coomassie brilliant blue G250 (CBB-G250), where the electrochemical signal of CBB-G250 was used to monitor the methylation event. After the hybrids of DNA S1 and DNA S2 were treated with M. *Sss*I MTase in the presence of *S*-adenosylmethionine, the MBD proteins were specifically conjugated to the methylation site of CpG dinucleotides, and then, the MBD proteins were stained with CBB-G250. The electrochemical signal of CBB-G250 increased linearly with increasing M. *Sss*I MTase concentration in the range from 0.1 to 40 unit/mL. Furthermore, the inhibition investigation demonstrates that fisetin and chlorogenic acid can inhibit the M. *Sss*I MTase activity with the IC_{50} value of 153.12 and 137.07 μM, respectively. Therefore, we think that this study may provide a sensitive platform for screening of DNA MTase inhibitors.

1. Introduction

Epigenetics is defined as heritable changes in gene expression that are not caused by changes in DNA sequence (Holliday, 1987). It mainly contains three systems, DNA methylation, RNA-associated silencing and histone modification (Egger et al., 2004). Among them, DNA methylation is the best known epigenetic marker. Up to now, DNA methylation at C-5 position of cytosine within CpG dinucleotides has been proved to be related to a wide range of biological phenomena, including inactivation of microRNA genes (Lujambio et al., 2007; Toyota et al., 2008), genomic imprinting (Li et al., 1993), X chromosome inactivation (Csankovszki et al., 2001) and tissue-specific gene expression (Bartolomei and Tilghman, 1997). More importantly, aberrant DNA methylation patterns have also been linked to certain genetic diseases and tumors, such as renal carcinoma (Herman et al., 1994), retinoblastoma tumor (Ohtani-Fujita et al., 1993), thyroid tumor (Boltze et al., 2003) and breast cancers (Miyamoto et al., 2005). It is well known that DNA methylation is induced by DNA methyltransferase (MTase), which can transfer methyl to C-5 position of cytosine residue of DNA from the methyl donor of *S*-adenosylmethionine (SAM). Therefore, the activity of MTase will influence DNA methylation level. It has also been reported that the MTase activity can be inhibited in the presence of inhibitors, such as 5-azacytidine (Christman, 2002), 5-aza-2′-deoxycytidine (Christman, 2002), procaine (Tada et al., 2007), caffeic acid (Lee and Zhu, 2006) and chlorogenic acid (Lee and Zhu, 2006). Therefore, it has great significance to develop a simple, rapid and specific method for methylation detection, MTase activity evaluation and inhibitor screening.

Electrochemical techniques have the advantages of simple operation, cheap instrument, time-saving, high sensitivity and selectivity. Several electrochemical methods have been developed for DNA methylation detection and DNA MTase assay (Su et al., 2012). Jiang's group proposed a novel electrochemical biosensor for activity assay of DNA methyltransferase based on methylation-sensitive cleavage, which activated a primer for terminal transferase-mediated extension of biotinylated dUTP followed by sensitive detection via enzymatic amplification (Wu et al., 2012). Liu et al. (2011) investigated another method for the detection of the genomic DNA methylation level based on the M. *Sss*I methylase-*Hpa*II endonuclease interaction system. He et al. (2011) developed a signal-on electrochemical assay for detection of Dam MTase activity based on DNA-functionalized gold nanoparticles amplification coupled with enzyme-linkage

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reactions. These methods showed high sensitivity and good selectivity. More importantly, these methods were PCR-free and nonradioactive.

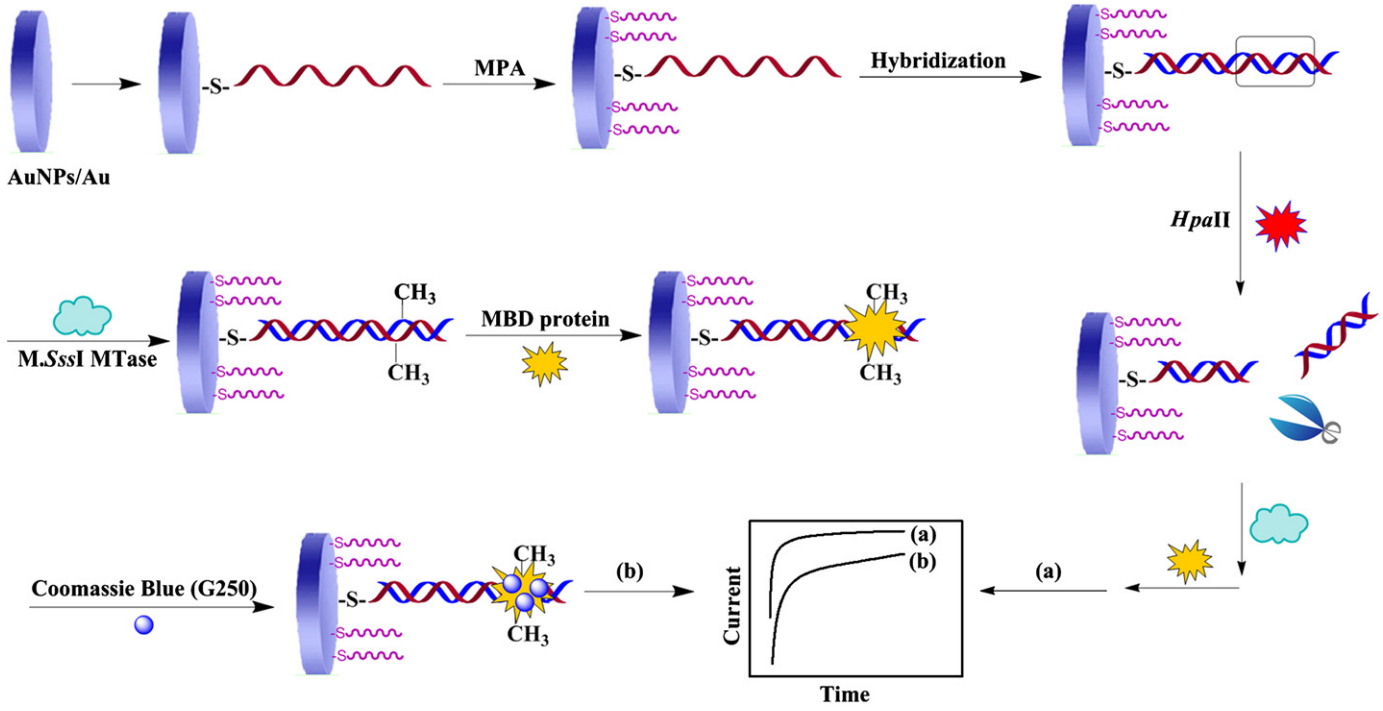
It has been reported that DNA methylation sites can be specifically recognized by a family of protein factors that contain conserved methyl-CpG binding domains (MBDs) (Lewis et al., 1992; Meehan et al., 1989). So far, there are at least five MBD family members characterized in mammals, MeCP2, MBD1, MBD2, MBD3, and MBD4 (Hendrich and Bird, 1998; Wade et al., 1999). Among them, MBD1, MBD2 and MBD4 have been shown to preferentially bind a symmetrically methylated CpG motif (Cross et al., 1997; Lewis et al., 1992; Suter et al., 2010). Therefore, MBDs may be used as specific analytical tools for detection of DNA methylation and assay of MTase activity.

In this work, we develop a novel electrochemical method for assay of DNA methylation and DNA MTase activity using the specific MBD protein without bisulfite treatment or other enrichment steps. The significant limitation of all bisulfite-based and enrichment-based approaches was the complicated operation process, the rigorous control, the low sensitivity and the duration of bisulfite treatment (Hu and Zhang, 2011; Kato et al., 2011; Pan et al., 2010; Wan et al., 2007; Yu et al., 2010). As seen in Scheme 1, this strategy is based on the amperometric response of electroactive molecule of Coomassie brilliant blue G250 (CBB-G250), which was specifically adsorbed on the MBD protein after the duplex DNA was methylated by M. *Sss*I MTase and then digested by *Hpa*II restriction endonuclease. The M. *Sss*I MTase can transfer the methyl to C-5 position of cytosine in the CpG region of double-stranded DNA from SAM and the *Hpa*II restriction endonuclease can identify the duplex symmetrical sequence of 5′-CCGG-3′ and catalyze the digestion of double-stranded DNA between the unmethylated cytosines. After the methylation event, the *Hpa*II digestion is blocked and the MBD proteins can undergo specific conjugation on the symmetrically methylated cytosine in duplex sequence of 5′-CCGG-3′. Then, the MBD protein specifically recognize and bond the methylated CpG region. The CBB-G250 can easily and firmly conjugate with protein through van der Waals forces, and it is hard to wash away from protein after conjugation. More importantly, CBB-G250 is an electroactive molecule and exhibits an oxidation signal, which has been proved in this work. Therefore, the oxidation signal of CBB-G250 is used to evaluate the methylation level, and the electrochemical signal change after *Hpa*II digestion is related the methylation status, which reflects the M. *Sss*I MTase activity. Based on them, an electrochemical assay is established for the evaluation of DNA methylation level and MTase activity. Moreover, because the aberrant DNA methylation normally contacts with various diseases, the screening of MTase inhibitors is very important in pharmacology and diseases treatment. In this work, we also investigate several reported compounds as MTase inhibitor on the inhibition activity towards M. *Sss*I MTase.

2. Experimental

2.1. Reagents and materials

3-Mercaptopropionic acid (MPA) was purchased from Alfa Aesar (Lancashire, England). Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3\,H_2O$), CBB-G250, tris(hydroxymethyl)aminomethane (Tris), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), fisetin and chlorogenic acid were purchased from Aladdin (Shanghai, China). Catechol-*O*-methyltransferase (COMT) was purchased from Sigma-Aldrich (St. Louis, USA). CpG methyltransferase M. *Sss*I, 10 × NEBuffer 2 and 200 × SAM (32 mM) were purchased from New England BioLabs (Ipswich, MA). Restriction endonuclease *Hpa*II and 10 × Buffer Tango™ were from Fermentas (Maryland, USA). M. *Sss*I MTase and *Hpa*II endonuclease were diluted to required concentration according to the manufacturer's recommendations with dilution buffer. The synthetic oligonucleotides were purchased from Shanghai Sangon Biotechnology Co. (Shanghai, China) and used without further purification. Their base sequences are as follows: thiol-capped probe DNA (DNA S1), 5′-SH-$(CH_2)_6$-TAG TGT GAT GTC ACC TAG TTG ACC TTC CGG AT-3′;



Scheme 1. Schematic representation of the developed method for detection of DNA methylation and assay of M. *SssI* MTase activity.