



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. SssI* 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. SssI* 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. SssI* MTase activity with the IC₅₀ value of 153.12 and 137.07 μ M, respectively. Therefore, we think that this study may provide a sensitive platform for screening of DNA MTase inhibitors.

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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. SssI* methylase-*HpaII* 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.

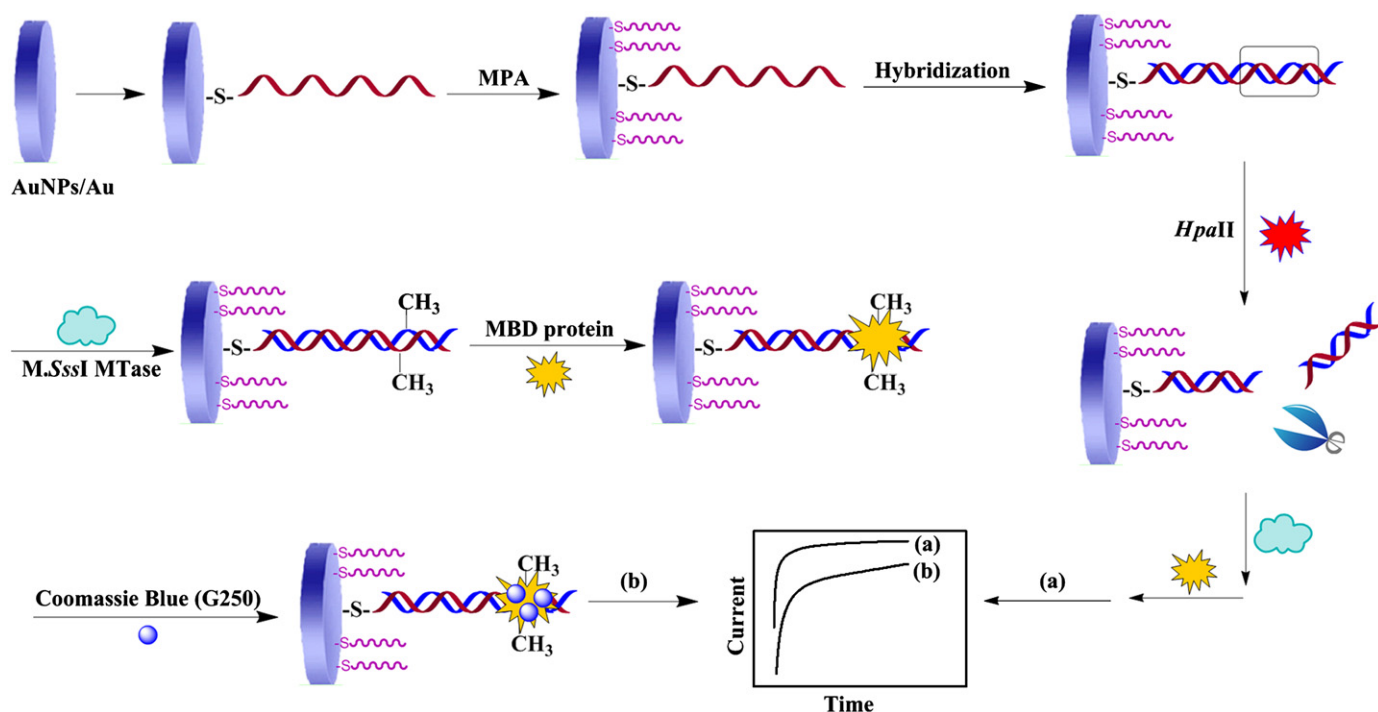
In this work, we develop a novel electrochemical method for assay of DNA methylation and DNA MTase activity using the specific MBD protein without bisulfite treatment or other enrichment steps. The significant limitation of all bisulfite-based and enrichment-based approaches was the complicated operation 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. SssI* MTase and then digested by *HpaII* restriction endonuclease. The *M. SssI* MTase can transfer the methyl to C-5 position of cytosine in the CpG region of double-stranded DNA from SAM and the *HpaII* 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 *HpaII* 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 *HpaII* digestion is related the methylation status, which reflects the *M. SssI* 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. SssI* MTase.

2. Experimental

2.1. Reagents and materials

3-Mercaptopropionic acid (MPA) was purchased from Alfa Aesar (Lancashire, England). Hydrogen tetrachloroaurate trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), CBB-G250, tris(hydroxymethyl)amino-methane (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. SssI*, 10 × NEBuffer 2 and 200 × SAM (32 mM) were purchased from New England BioLabs (Ipswich, MA). Restriction endonuclease *HpaII* and 10 × Buffer Tango™ were from Fermentas (Maryland, USA). *M. SssI* MTase and *HpaII* 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-(CH₂)₆-TAG TGT GAT GTC ACC TAG TTG ACC TTC CCG AT-3';



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

target DNA (DNA S2), 5'-ATC CCG AAG GTC AAC TAG GTG ACA TCA CAC TA-3'; one-base mismatched DNA (DNA S3), 5'-ATC CTG AAG GTC AAC TAG GTG ACA TCA CAC TA-3'. The synthesized oligonucleotides were diluted in the TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) to desired stock concentrations and stored at -20°C according to the manufacturer's instructions.

The buffer solutions employed in this study are as follows. Probe immobilization buffer: 10 mM Tris-HCl, 1.0 mM EDTA, 1.0 M NaCl, and 1.0 mM TCEP (pH 7.0). DNA hybridization buffer: 10 mM Tris-HCl, 1.0 mM EDTA, and 1.0 M NaCl (pH 7.4). MBD protein immobilization buffer: 0.1 M phosphate buffered saline (PBS, pH 7.0) and 5% glycerol. $1\times$ NEBuffer 2: 50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl_2 , 1 mM dithiothreitol (pH 7.9). $1\times$ Buffer TangoTM: 33 mM Tris-acetate, 10 mM magnesium acetate, 66 mM potassium acetate and 0.1 mg/mL BSA (pH 7.9). Electrochemistry determination buffer: 0.1 M PBS (pH 7.0). PBS was prepared by mixing the stock solution of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 , and the pH was adjusted by NaOH or HCl. All reagents were analytically pure grade. The double distilled deionized water used was autoclaved (the ohmic resistivity was 1.16 M Ω).

2.2. Protein expression

Recombinant plasmids pET30(b+)-1xMBD were received from Adrian Bird's lab (Edinburgh, UK). The MBD protein expression was performed according to the previous report (Yu et al., 2010). Protein separation and purification were carried out at AKTA FPLC system (GE, Sweden) with Ni-NTA column. The degree of purification was estimated by SDS-PAGE, and protein concentration was measured via the Bradford assay. The MBD protein was diluted to the required concentration with 0.1 M PBS (pH 7.0) and stored at -80°C before use.

2.3. Preparation of AuNPs modified electrode

An Au disc electrode (2 mm in diameter, Gaoss Union Instrument) was used as substrate electrode. Prior to use, the electrode was polished sequentially with 0.05 μm alumina slurries to create a mirror-like surface, and then sonicated with absolute ethanol and double distilled deionized water for 3 min, respectively. After dried with nitrogen blowing, the gold electrode was dipped into $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ (7:3, v/v) solution for 10 min. Then, it was washed successively with double distilled deionized water in an ultrasonic bath. The electrode was then subjected to electrochemical pretreatment by consecutive potential cycling in 0.2 M H_2SO_4 solution within a potential window between -0.2 and 1.5 V at a scan rate of 100 mV s^{-1} until a characteristic Au peak was obtained. The electrodeposition of gold nanoparticles (AuNPs) on the pretreated Au electrode was conducted at -0.2 V in 3.0 mM HAuCl_4 and 0.1 M KNO_3 solution for 200 s under stirring. After that, the electrode was washed with double distilled deionized water and dried under N_2 blowing.

2.4. Biosensor preparation

The immobilization of thiolated probe DNA S1 on AuNPs modified Au electrode was performed by dripping 5 μL probe immobilization buffer containing 5.0×10^{-7} M DNA S1 on the electrode surface and maintained for 12 h at humidity condition (the relative humidity was 99%). Then, the modified electrode was rinsed with 10 mM Tris-HCl (pH 7.0) three times to remove the non-specific immobilized DNA molecule from the electrode surface. After that, 5 μL MPA (2.5 μM in probe immobilization buffer) was dripped on the electrode surface to further eliminate the

non-specifically immobilized DNA and obtain well-aligned DNA monolayers.

Prior to hybridization, the modified electrode was rinsed with 5 mL of 10 mM Tris-HCl (pH 7.0). After dried under N_2 blowing, 5 μL DNA hybridization buffer containing 5.0×10^{-7} M different target DNA (complementary or non-complementary) was dripped on the modified electrode surface and kept at 37°C for 2 h. And then, the hybridized electrode was rinsed with 10 mM Tris-HCl (pH 7.0) and dried with N_2 blowing.

The electrode was then treated with M. SssI MTase to transfer the methyl from SAM to C-5 position of cytosine in CpG region of hybridized DNA. To achieve it, the electrode was incubated with 5 μL $1\times$ NEBuffer 2 containing 160 μM SAM and 40 unit/mL of M. SssI MTase at 37°C for 2 h. After rinsed with 10 mM Tris-HCl (pH 7.0), the modified electrode surface was further treated with 5 μL of 80 $\mu\text{g/mL}$ MBD protein solution for another 2 h. Finally, 5 μL CBB-G250 solution was dripped on the modified electrode surface after the electrode was rinsed with 10 mM Tris-HCl (pH 7.0) and incubated for 30 min at ambient temperature. After removing any remaining CBB-G250 adsorbed nonspecifically by rinsing with 0.1 M PBS (pH 7.0), the electrode was subjected to electrochemical measurement.

2.5. HpaII endonuclease digestion

The hybridized DNA digestion by HpaII endonucleases was performed at 37°C for 2 h by dripping 5 μL $1\times$ Buffer TangoTM containing 40 unit/mL HpaII on the electrode surface. After digestion, the electrode was thoroughly rinsed with 10 mM Tris-HCl (pH 7.0) and dried under N_2 blowing.

2.6. Inhibition the activity of M. SssI MTase

To study the inhibition effects of fisetin and chlorogenic acid on the M. SssI activity, the methylation of S1/S2 hybrid was performed at 37°C in $1\times$ NEBuffer 2 (pH 7.9) containing 160 μM SAM, 400 U/mL M. SssI MTase, 40 U/mL COMT and various concentration of the inhibitors (from 0 to 300 μM) (Lee and Zhu, 2006). The inhibition efficiency (%) is estimated as follows:

$$\text{Inhibition (\%)} = \frac{I_2 - I_3}{I_2 - I_1} \times 100\%$$

where I_1 is the current of the hybrid of S1/S2 after treated with M. SssI MTase and MBD protein, I_2 is the current of the hybrid of S1/S2 after treated with M. SssI MTase, MBD protein and CCB-G250, and I_3 is the inhibited current.

2.7. Electrochemical detection

Electrochemical experiments were performed with CHI660C electrochemical workstation (Austin, Texas, USA). Experiments were performed in 3-electrode geometry. A bare Au or modified Au electrode (Gaoss Union, 2 mm in diameter) was used as working electrode. A saturated calomel electrode (SCE) and a platinum wire were used as the reference electrode and auxiliary electrode, respectively. All the measurements were carried out without degassing the electrolyte.

Electrochemical impedance spectroscopy (EIS) was carried out in 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ (1:1, molar ratio) solution containing 0.1 M KCl as the supporting electrolyte. The frequency was ranged from 10^{-1} to 10^5 Hz. The applied potential is the formal potential. The chronoamperometry (CA) was employed to sense the specific adsorption of CBB-G250, and to characterize the methylation efficiency. The parameters are as follows. Initial potential, 0.2 V; high potential, 0.55 V; low potential, 0.2 V; pulse width, 100 s; sampling interval, 0.01 s; quiet time, 2 s.

3. Results and discussion

3.1. Characterization of different self-assembly process by EIS

The self-assembly process was characterized by EIS. As shown in Fig. 1, the bare Au electrode showed an electron transfer resistance (R_{et}) of about 536.5 Ω (curve a). After AuNPs was electrodeposited on the Au electrode surface, the R_{et} value decreased significantly and only a straight line was obtained in the selected frequency region (curve b). However, the R_{et} value increased to 159.1 Ω after the thiol-modified probe DNA S1 was assembled on the electrode surface (curve c), causing by the electrostatic repulsion between the negatively charged deoxyribose-phosphate backbone of probe oligonucleotides and $\text{Fe}(\text{CN})_6^{3-/4-}$. It demonstrated that the probe was successfully assembled on the electrode surface. After hybridized with complementary DNA S2, the R_{et} value showed an obvious increase (377.9 Ω , curve d), implying the formation of double helix structure of DNA S1 and DNA S2. When the double helix DNA was treated with M. SssI MTase and MBD protein, the R_{et} value increased to 632.6 Ω (curve e), indicating the conjugation of MBD protein on the CpG methylation region was achieved after DNA methylation catalyzed by M. SssI MTase. Because CBB-G250 has two sulfo groups in its molecule structure, it is charged by negative charge. Therefore, the R_{et} value further increased after the MBD protein was stained by CBB-G250 (curve f). Curve g was the EIS of the electrode after the double helix DNA was treated successively by *HpaII* endonucleases, M. SssI MTase, MBD protein and CBB-G250. The R_{et} value (287.6 Ω) was between curve c and curve d. It can be attributed to the digestion effect of *HpaII* endonucleases towards the unmethylated double helix DNA, which made the DNA scissored to two fragments, and the following treatments were blocked.

3.2. Electrochemical activity of CBB-G250

The electrochemical activity of CBB-G250 was proved in this work (see Supplementary material). CBB-G250 dye is known to be used as protein stain in polyacrylamide gel electrophoresis gels for protein analysis (Dong et al., 2011; Georgiou et al., 2008). This kind of dye can bind to proteins via physical adsorption to aromatic amino acids and other amino acids by van der Waals forces, and this interaction is tight and rapid. Considering the stain property and electrochemical activity of CBB-G250, we employed CBB-G250 to stain the BMD protein and the electrochemical signal of CBB-G250 was used to monitor the DNA methylation and MTase activity.

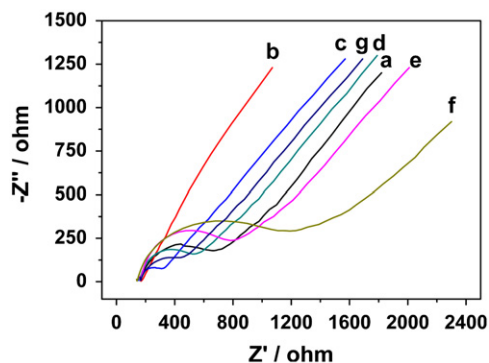


Fig. 1. Nyquist plots of the bare Au electrode with different assembly processes. (a) Au electrode. (b) AuNPs modified Au electrode. (c) Probe DNA S1 assembled on the modified electrode. (d) Probe DNA 1 hybridized with target DNA S2. (e) MBD proteins were conjugated with methylation sites after the S1/S2 hybrids were treated with M. SssI MTase. (f) MBD protein was stained with CBB-G250. (g) The S1/S2 hybrids were first treated with *HpaII* endonucleases, then M. SssI MTase, MBD protein and CBB-G250 successively.

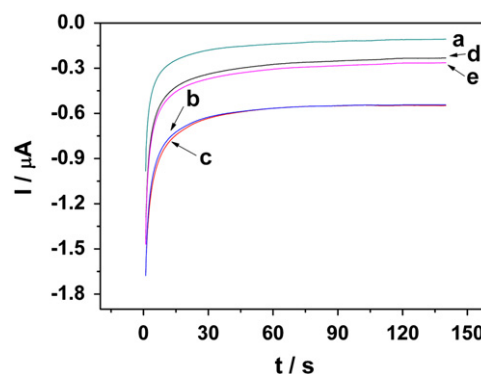


Fig. 2. Amperometric responses of the biosensor with different treatment processes in 0.1 M PBS of pH 7.0. (a) The MBD proteins were conjugated with methylated site. (b) The S1/S2 hybrids were treated with M. SssI MTase firstly, and then digested with *HpaII* endonuclease, finally, treated with MBD protein and CBB-G250 successively. (c) The S1/S2 hybrids were treated with M. SssI MTase, MBD protein and CBB-G250 successively. (d) The probe DNA S1 hybridized with target DNA S2. (e) The S1/S2 hybrids were treated with *HpaII* endonuclease firstly, and then digested with M. SssI MTase, finally, treated with MBD protein and CBB-G250 successively.

Due to the high sensitivity of amperometry, chronoamperometry was selected to investigate the electrochemical behavior of working electrode before and after MBD protein stained with CBB-G250. As shown in Fig. 2, after the hybridized DNA (S1/S2) was successively treated with M. SssI MTase, MBD protein and CBB-G250, a big current response was obtained (curve c) compared with the current response obtained after the hybridized DNA (S1/S2) was treated with (curve a) and without (curve d) M. SssI MTase and MBD protein under the same conditions. The current improvement should be caused by the adsorption of CBB-G250 on the MBD protein. It indicated that the CpG region of the hybridized DNA was successfully methylated by M. SssI MTase in the presence of methyl donor of SAM and the specifically conjugated MBD protein was confirmatively stained by CBB-G250. For further proving it, the double helix DNA was first treated with *HpaII* endonuclease, which can specifically digest the double helix region of 5'-CCGG-3' and block the methylation. Then, the digested double helix DNA was further treated successively with M. SssI MTase, MBD protein and CBB-G250 (curve e). The current response was only a little higher than that at curve d. It might be ascribed to the digestion effect on the double helix DNA, which blocked the methylation event, and caused the failure on MBD protein and CBB-G250 immobilization. However, if the double helix DNA was first methylated by M. SssI MTase, then treated with *HpaII* endonuclease, MBD protein and CBB-G250 (curve b), it can be concluded that the digestion effect can be blocked by the methylation of CpG region, producing a similar current response to curve c.

3.3. Assay of M. SssI MTase activity

For quantitative analysis of the activity of M. SssI MTase activity, the effect of M. SssI MTase concentration and methylation time on the methylation level was investigated by chronoamperometry.

For M. SssI MTase concentration, the double helix DNA on the electrode surface was first methylated with different concentration of M. SssI MTase for 2 h, then, the methylated DNA was treated successively with *HpaII* endonuclease, MBD protein and CBB-G250. Finally, the current response of CBB-G250 was recorded by chronoamperometry. As seen in Fig. 3, the current response increased gradually with increasing the concentration of M. SssI concentration. It indicated that the high concentration of M. SssI MTase can catalyze more double helix DNA to be methylated. As shown in the inset of

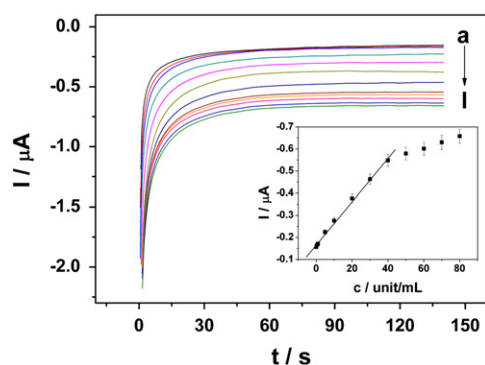


Fig. 3. The effects of the concentration of *M. SssI* MTase on the amperometric response of the biosensor. The concentrations of *M. SssI* for the curves from (a) to (l) are 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20, 30, 40, 50, 60, 70 and 80 unit/mL. Inset: the plot of the amperometric current vs. the concentration of *M. SssI* MTase.

Fig. 3, the current increased linearly in the low concentration range and showed a tendency to achieve a plateau at high concentration, because the double helix DNA could be almost completely methylated at a high concentration level of *M. SssI* MTase, and the immobilization amount of MBD protein and CBB-G250 achieved the saturation state. As a result, the current response of CBB-G250 increased slowly even though the concentration of *M. SssI* MTase continued to increase. More importantly, the digestion of double helix DNA by *HpaII* endonuclease could be blocked after the completely methylation. The linear regression equation can be expressed as $I = -0.0098c - 0.17$ (μA , U/mL, $R = 0.9951$) with the concentration ranging from 0.1 to 40 U/mL. The detection limit was 0.04 unit/mL ($S/N = 3$), which was better than previous reports lower than that obtained using methylation-responsive DNAzyme methods (6 unit/mL) (Li et al., 2010), cross-linking AuNPs aggregation (2.5 unit/mL) (Song et al., 2009), gold nanoparticles coupled with enzyme-linkage reactions (0.3 unit/mL) (Liu et al., 2009), DNA-functionalized gold nanoparticles amplification coupled with enzyme-linkage reactions (He et al., 2011), electrochemical method with ferrocene acetic acid as electroactive label (0.1 unit/mL) (Liu et al., 2011). However, the detection limit was higher than that obtained using electrogenerated chemiluminescence biosensing method (0.02 unit/mL) (Li et al., 2012).

Fig. 4 showed the effect of methylation time on the current response of CBB-G250. For this investigation, 5 μL methylation solution (containing 40 U/mL *M. SssI* MTase, 160 μM SAM, 1 $\times$ NEBuffer 2) was dripped on the double helix DNA modified electrode surface, and the methylation time was ranged from 0 to 180 min. Then, the electrode was treated successively with *HpaII* endonuclease, MBD protein and CBB-G250. Finally, the response was recorded by chronoamperometry. The current response increased linearly with extending methylation time from 0 to 120 min, and then gradually leveled off. With extending the treatment time of *M. SssI* MTase, the amount of methylated double helix DNA increased gradually, which can lead to the increase of the immobilization amount of MBD protein and CBB-G250. However, when the treatment time was extended to 120 min, the methylation towards double helix DNA trended to complete.

3.4. Precision, repeatability and selectivity

The precision, repeatability and selectivity of the proposed method were also evaluated. According to the previous report (Liu et al., 2011), the assay precision was estimated with the slopes of calibration plots obtained from three independent assay systems. The relative standard deviation (RSD) of these slopes was 5.87%, indicating that the developed method could be applied to quantitatively analyze *M. SssI* MTase activity and content in samples.

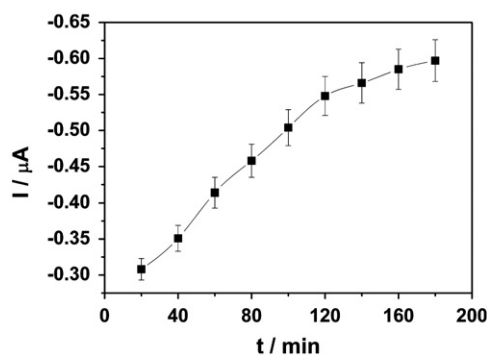


Fig. 4. Dependence of amperometric current of the biosensor on the methylation time.

The repeatability assay was performed with the *M. SssI* MTase concentration of 50 U/mL for five detection based on freshly fabricated electrode. The RSD was 6.49%, implying an acceptable repeatability. The intra-assay coefficient of variation was also measured with 6.51% using 20 U/mL *M. SssI* MTase for five replicates measurements. The selectivity of the proposed method was verified by testing 0.5 μM single-base mismatched DNA S3, where the mismatched base was located at the CpG region. The current response was closed to the current obtained at the probe DNA S1 modified electrode, indicating that the methylation catalyzed by *M. SssI* MTase failed due to the base mismatch and CBB-G250 was not adsorbed on the electrode surface. This result proved that the developed method had good selectivity.

3.5. Inhibition investigation

DNA-specific methylation is known to be associated with the inactivation of various pathways involved in the tumorigenic process, including DNA repair (Li et al., 1993), cell cycle regulation (Li et al., 1992), inflammatory/stress response (Beard et al., 1995) and apoptosis (Esteller, 2002). DNA methylation is catalyzed by specific DNA MTase, using SAM as methyl donor. One important factor in the methylation process is the DNA MTase activity. Therefore, the research for inhibiting DNA MTase activity has attracted more and more attentions because it can provide a broad spectrum of therapeutic applications (Bender et al., 1998; Creusot et al., 1982; Tada et al., 2007; Wilson et al., 1983). It has been reported that several tea catechins and bioflavonoids could inhibit the DNA methylation catalyzed by *M. SssI* DNA MTase (Lee et al., 2005; Lee and Zhu, 2006). As known that COMT can catalyze the *O*-methylation reaction of several tea catechins and bioflavonoids. This catalytic reaction can not only consume SAM, but also form equimolar amounts of *S*-adenosyl-L-homocysteine, which is the demethylated SAM and also a feedback inhibitor of various SAM-dependent methylation processes (Lee et al., 2005; Lee and Zhu, 2006). In order to confirm the validity of this developed method on inhibitor screening, we selected fisetin (bioflavonoid) and chlorogenic acid (tea catechin) as model complex to investigate the inhibition effect on *M. SssI* MTase activity in the presence of COMT. As can be seen in Fig. 5, the inhibition efficiency increased with increasing the concentration of fisetin (Fig. 5A) and chlorogenic acid (Fig. 5B), implying that the inhibition of *M. SssI* activity was dose-dependent. The maximum inhibition was about 63.66% and 60.73% for fisetin and chlorogenic acid with the inhibitor concentration of 300 μM , respectively. The IC_{50} values were 153.12 and 137.07 μM for fisetin and chlorogenic acid, respectively. These results showed that the developed assay method has the potential ability to screen the inhibitors of DNA MTase.

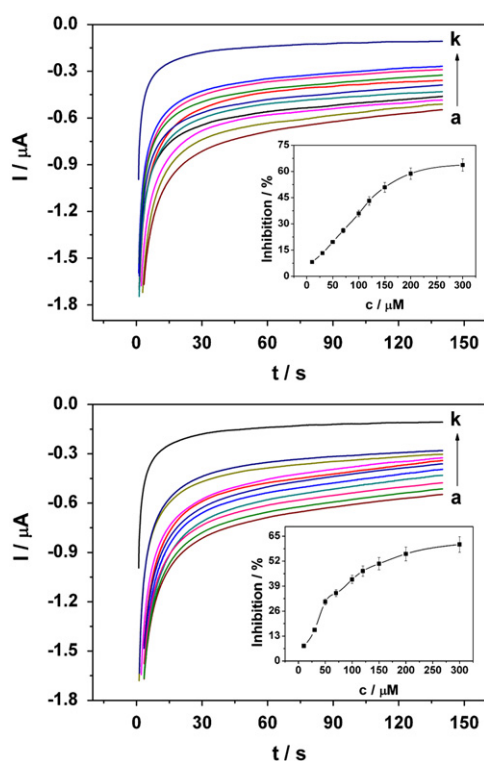


Fig. 5. The amperometric response of the biosensor after the M. Sssl MTase activity was inhibited with different concentrations of fisetin (A) and chlorogenic acid (B). The concentrations of inhibitor from (a) to (j) are 10, 30, 50, 70, 100, 120, 150, 200 and 300 μM . Curve (f) is the blank response, that is the S1/S2 hybrids were treated with M. Sssl MTase, HpaII endonuclease and MBD protein successively. Inset: the relationship between inhibition efficiency and inhibitor concentration.

4. Conclusion

In summary, we developed a sensitive biosensor for detection of DNA methylation and assay of DNA MTase activity. This method takes advantages of the specific CpG methylation binding protein of MBD protein and the electrochemical activity dye of CBB-G250. Compared with traditional methods, this method does not need expensive and sophisticated instrument, skilled operator, bisulfite treatment, methylated nucleotide labeling or PCR amplification. More importantly, the consume time of this method was few than bisulfite-based methods. This simple method can be applied to screen MTase inhibitor, which might be benefit to discover anti-cancer drugs.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bios.2012.09.010>.

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