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Short communication

DNA methyltransferase activity assay based on visible light-activated photoelectrochemical biosensor

Yunlei Zhou, Zhenning Xu, Mo Wang, Bing Sun, Huanshun Yin*, Shiyun Ai*

College of Chemistry and Materials Science, Shandong Agricultural University, Daizong Street 61, Taian, Shandong 271018, PR China

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ABSTRACT

DNA methylation has important roles in gene regulation and relates to some diseases, especially cancers. Because DNA methylation is catalyzed by DNA methyltransferases (MTase), it is important to detect the activity of DNA MTase. In this work, we developed a novel visible light-activated photoelectrochemical (PEC) biosensor for DNA MTase activity assay, whereby bismuth oxyiodide (BiOI) nanoflake was synthesized as photoactive electrode material, M. SssI MTase as methylation reagent and methyl binding domain protein (MBD1 protein) as methylation recognition element. After cytosine methylation event occurred at the site of 5′-CG-3′, it could be probed by MBD1 protein and this protein could be combined tightly with methylated cytosine, which would lead to a decreased photocurrent due to the hindrance towards electron donor transferring to electrode surface by huge-volume protein. The decreased photocurrent was proportional to M. SssI MTase concentration from 0.1 to 50 unit/mL with the detection limit of 0.035 unit/mL (S/N=3). This detection limit was lower than that in some previous reports. This PEC biosensor showed high selectivity and good reproducibility for M. SssI MTase assay. Moreover, this method was successfully applied also to screen DNA MTase inhibitors, indicating that this PEC biosensor could be an alternative platform in anti-cancer pharmaceuticals discovery.

1. Introduction

DNA methylation is a major epigenetic modification, which plays a significant regulatory role in both prokaryotes and eukaryotes (Robertson, 2005). DNA methylation is catalyzed by DNA methyltransferases (MTase), which can recognize specific DNA sequences and transfer a methyl group from methyl donor of S-adenosyl-L-methionine (SAM) to cytosine at C-5 position or adenine at the 6-amino group (Song et al., 2009). Aberrant DNA methylation can influence the interaction between DNA and protein or even alter gene expression. Therefore, it has been associated with many diseases, especially cancers (Robertson, 2005). Research on the inhibition screening on DNA MTase activity may offer useful drug information on cancer therapy. Thus, development of a sensitive method to measure the activity of DNA MTase is required for the early diagnosis of cancer.

Photoelectrochemical (PEC) assay is a newly developed analytical method with high sensitivity and low background current due to the separation of excitation source and detection signal. Various PEC sensors have been fabricated and used in the detection of DNA (Zhang et al., 2013), DNA damage (Zhang et al., 2012), enzyme (Zhang et al., 2011), protein (Wang et al., 2009), small molecule (Gong et al., 2012) and metal ion (Zhang and Guo, 2012). However, no work has been done for assay of DNA MTase activity based on PEC technique. For developing a visible light-activated photoelectrochemical biosensor, BiOI attracted our attention due to the smallest bandgap (~1.8 eV) and strong absorption in the visible light region (the absorption edge is ~680 nm). Though BiOI has showed photocatalytic performance under sunlight irradiation, only a few reports described its application in PEC biosensor. Gong et al. developed a sensitive and selective biosensor for the PEC detection of organophosphate pesticides using BiOI as photoactive material with detection limit of 0.04 ng/mL (Gong et al., 2012).

Methyl binding domain (MBD) protein can recognize specifically symmetrical CpG methylation in double-stranded DNA (dsDNA) with high affinity (Inomata et al., 2008). Up to now, MBD protein has been used for methylated DNA precipitation (Zou et al., 2007) or direct detection of methylated DNA (Hiraoka et al., 2012). The MBD-based assay does not require bisulfite treatment and can target all genomic regions. Based on the advantages of PEC detection and MBD protein, we developed a novel photoelectrochemical (PEC) method for assay of DNA MTase activity and screening of inhibitor using BiOI as photoactivity material. As shown in Scheme 1, the dsDNA was first immobilized on gold nanoparticles (AuNPs) and BiOI modified ITO electrode. Then, the dsDNA was methylated by M. SssI MTase and was captured by MBD1 protein, which resulted in a decreased photocurrent due to steric hindrance of MBD1 protein, blocking the transfer of electron donor of ascorbic

* Corresponding authors. Tel.: +86 538 8249248; fax: +86 538 8247660.
E-mail addresses: yinhs@sdau.edu.cn (H. Yin), ashy@sdau.edu.cn (S. Ai).

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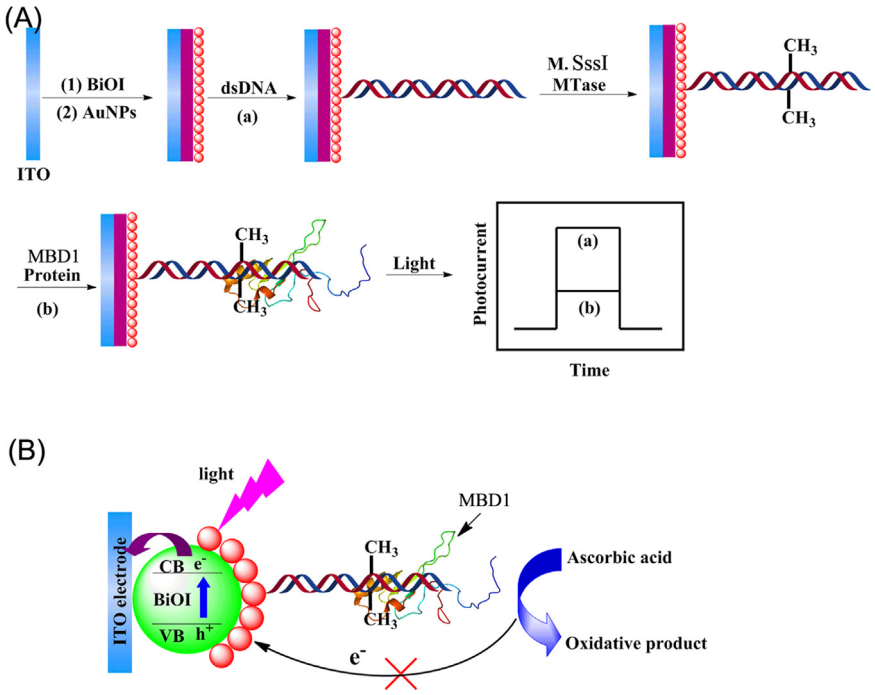

Scheme 1. (A) Schematic diagram of the PEC biosensor construction process. (B) Photocurrent generation and decrease mechanism of the PEC biosensor.

acid to electrode surface. The decrease of the photocurrent is related to the methylation level of dsDNA, and the latter is also related to the activity of M. SssI MTase. Therefore, the activity of DNA MTase could be detected according to the decreased photocurrent.

2. Experimental section

2.1. Reagents and instruments

MBD1 protein was recombined according to our previous work (Yin et al., 2013b). M. SssI MTase and restriction endonuclease HpaII were purchased from New England BioLabs (Ipswich, MA, USA) and Fermentas (Maryland, USA), respectively. Chloroauric acid ($HAuCl_4$), ascorbic acid (AA), trisodium citrate, tris(hydroxymethyl)aminomethane (Tris), tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 98%) and 5-fluorouracil were purchased from Aladdin (Shanghai, China). AuNPs and BiOI were synthesized according to a previous report (Liu and Lu, 2006; Yu et al., 2010) (see Supporting information). The synthetic oligonucleotides were purchased from Shanghai Sangon Biotechnology Co. (Shanghai, China) and the base sequences of oligonucleotides are as follows: thiol-capped probe DNA (DNA S1), 5′-SH-$(CH_2)_6$-TAG TGT GAT GTC ACC TAG TTG ACC TT**C CGG**-3′; target DNA (DNA S2), 5′-**CCG G**AA GGT CAA CTA GGT GAC ATC ACA CTA-3′. The synthesized oligonucleotides were diluted in a TE buffer (10 mM Tris–HCl and 1 mM EDTA, pH 8.0) to desired stock concentrations and stored at −20 °C. The buffer solutions employed in this study are listed in Supporting information. All reagents were of analytically pure grade. All of the solution and redistilled deionized water used were autoclaved.

PEC measurements were performed with a home-built PEC system. A 500 W Xe lamp equipped with an optical filter was used as the irradiation source to produce visible-light (light intensity was 20 mW/cm^2). The photocurrent was recorded on a CHI832A electrochemical workstation (CHI instruments, Austin, USA) with a three-electrode system in PBS (0.1 M, pH 7.0) containing 0.1 M AA, which was deaerated by nitrogen for 30 min before experiments. Electrochemical impedance spectroscopy (EIS) was carried out on a CHI660C electrochemical workstation (CHI instruments, Austin, USA). A transmission electron microscopy (TEM) image was taken with a JEOL-1200EX instrument (Japan). Powder X-ray diffraction (XRD) data were recorded at a scanning rate of (2θ) 0.02 deg S^{-1} using a Bruker D8-advance X-ray diffractometer at 40 kV and 40 mA for monochromatized Cu Kα (λ=1.5418 Å) radiation (Germany).

2.2. PEC biosensor fabrication

Before biosensor fabrication, the dsDNA was prepared first. A 20 μL DNA hybridization buffer containing 1 μM DNA 1 and DNA 2 was heated to 95 °C, followed by gradual cooling to room temperature. The obtained dsDNA solution was stored at −20 °C before use.

The ITO slices were used as the working electrode, which were cleaned by immersion in ethanol/NaOH mixed solution (v/v, 1:1) and acetone successively with sonication for 30 min, followed by washing copiously with double distilled deionized water and dried at 60 °C for 2 h. Then, 40 μL BiOI dispersion (3 mg/mL in double distilled deionized water) was dropped onto a piece of ITO slice with fixed area of 0.196 cm^2. After drying in air, the obtained BiOI/ITO electrode was rinsed with double distilled deionized water three times. Afterwards, 40 μL of AuNPs was further dropped on the electrode surface and dried under an infrared lamp, followed by rinsing with double distilled deionized water three times (the electrode was noted as AuNPs/BiOI/ITO). Subsequently, the electrode was incubated with 20 μL DNA immobilization buffer containing 1 μM thiol modified dsDNA for 12 h under humid conditions. The obtained electrode (noted as dsDNA/AuNPs/BiOI/ITO) was rinsed with 10 mM Tris–HCl (pH 7.4) three times to remove the un-immobilized dsDNA.

Methylation of dsDNA was performed at 37 °C by incubating dsDNA/AuNPs/BiOI/ITO for 2 h with 10 mM Tris–HCl buffer (pH 7.4) containing 160 μM SAM, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), and various concentrations of M. SssI MTase. Then, the treated electrode was rinsed with 10 mM Tris–HCl (pH 7.4) three times.

Restriction endonuclease HpaII digestion was performed at 37 °C by incubating the methylated electrode for 2 h with 10 mM

Tris–HCl buffer (pH 7.4) containing 50 unit/mL HpaII, 50 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), 200 μg/mL bovine serum albumin (BSA) and 50% glycerol. After digestion, the electrode was thoroughly washed with 10 mM Tris–HCl (pH 7.4) three times.

Finally, 20 μL MBD1 protein (0.01 mg/mL) was dropped on the electrode surface and incubated for 2 h under humid conditions at 37 °C. The obtained electrode (noted as MBD1/dsDNA/AuNPs/BiOI/ITO) was rinsed with 10 mM Tris–HCl (pH 7.4) three times.

2.3. Inhibition of M. SssI MTase activity

The inhibition effect could be quantitatively analyzed using photocurrent. For investigating the inhibition effects of 5-fluorouracil on the activity of M. SssI MTase, methylation of dsDNA was performed at 37 °C for 2 h in a 10 mM Tris–HCl buffer (pH 7.4) containing 160 μM SAM, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 50 unit/mL M. SssI MTase, and various concentrations of the inhibitors.

3. Results and discussion

3.1. Characterization of BiOI

The morphology and structure of the prepared BiOI were investigated by SEM, as shown in Fig. 1(A). The BiOI product was composed of a large quantity of relatively uniform plates with the length of $\leq$ 500 nm. Fig.1(B) shows the XRD pattern for BiOI. All the peaks for the BiOI sample were readily indexed to the tetragonal phase of BiOI (JCPDS Card No. 10-0445). No characteristic peaks of the other impurities were observed.

3.2. Biosensor characterization

The fabrication process of the PEC biosensor was characterized first by EIS and the results are shown in Fig. 1(C) (see Supporting information). The fabrication of the PEC biosensor was also monitored by photocurrent measurements. As shown in Fig. 1(D), a stronger photocurrent was obtained (curve b) after immobilization of BiOI nanoplates on ITO electrode, indicating the photoactivity of BiOI. However, the photocurrent decreased by about 13.58% for AuNPs immobilization (curve c). This decrease could be attributed to two factors. Firstly, the electron donated by AA was captured by AuNPs, which increased the recombination probability of photogenerated electrons and holes, and resulted in a decreased photocurrent. Secondly, there are many citrate anions adsorbed on AuNPs surface, which repelled the negative charge controlled AA. Subsequently, the photocurrent further decreased when dsDNA was self-assembled on electrode surface via Au–S bond (curve d), which could be explained by two factors. One was the steric-hindrance effect of the assembled dsDNA, which blocked the diffusion of electron donor of AA to electrode and resulted in an increased recombination rate of electron–hole pair of BiOI. The other was the electrostatic repulsion effect between dsDNA and AA. It is well known that the AA molecule was electronegative in pH 7.4 solution; thus the negative charged phosphate skeleton of dsDNA repelled AA and hampered the diffusion of AA to electrode. The photocurrent decreased significantly after the assembled dsDNA was incubated with M. SssI MTase, HpaII and MBD1 protein successively (curve e). The decreased photocurrent could be explained by the fact that the immobilized MBD1 protein partly hindered AA to BiOI surface. This also confirmed that the dsDNA was methylated and the cleavage was blocked. We think that our strategy could be applied to detect DNA MTase activity.

3.3. Detection performances

The photocurrent response of MBD1/dsDNA/AuNPs/BiOI/ITO upon visible light irradiation was found to be dependent on the immobilization amount of MBD1 protein, which was related to the methylation level of dsDNA. That is to say, the photocurrent response of the PEC biosensor depended on the concentration of DNA MTase, thus forming the detection basis. Fig. 2(A) depicts the photocurrent response of MBD1/dsDNA/AuNPs/BiOI/ITO upon visible light irradiation where the dsDNA was methylated by different

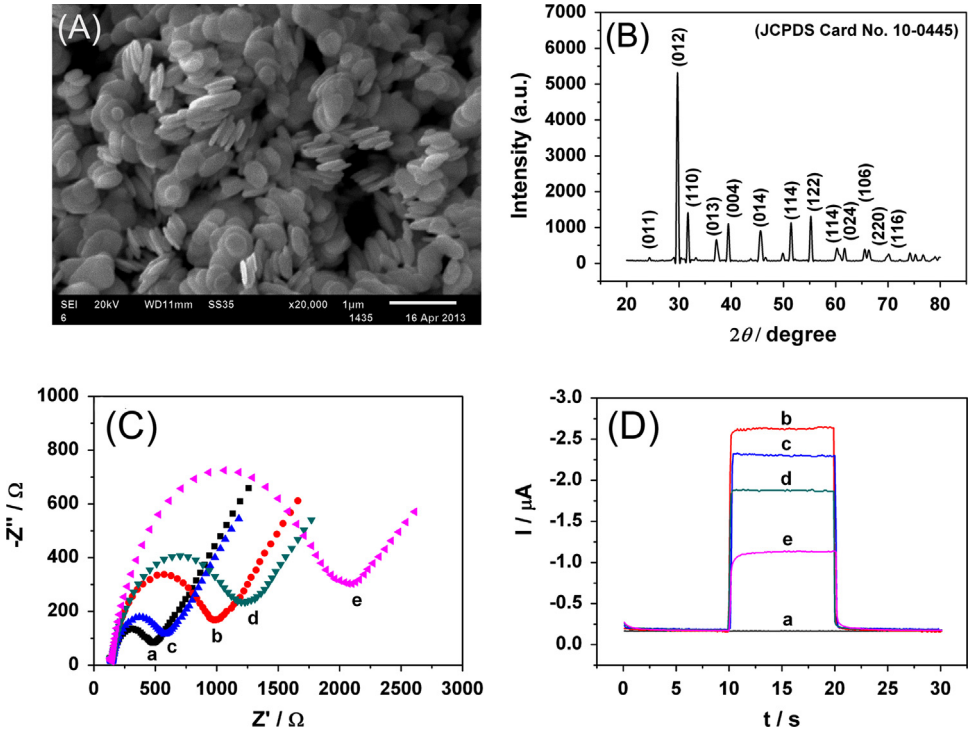

Fig. 1. SEM image (A) and XRD pattern (B) of BiOI. Nyquist diagrams (C) and photoelectrochemical response (D) of different electrodes. The applied potential was 0 V. (a) ITO, (b) BiOI/ITO, (c) AuNPs/BiOI/ITO, (d) dsDNA/AuNPs/BiOI/ITO, and (e) MBD1/dsDNA/AuNPs/BiOI/ITO.

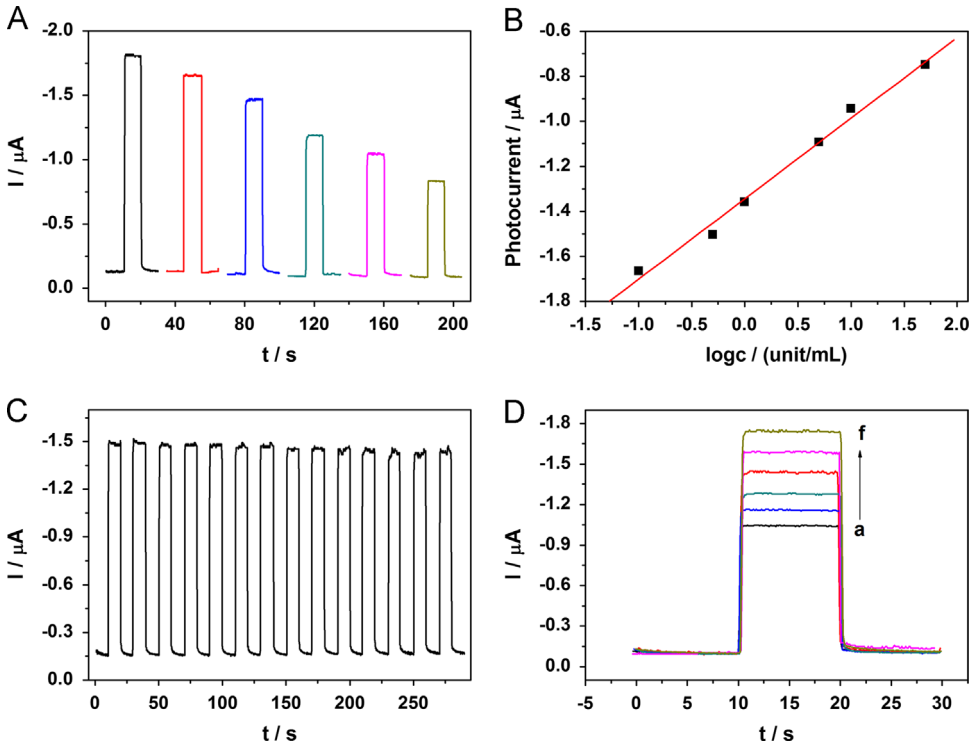

Fig. 2. (A) Photocurrent responses of the PEC biosensor after treatment with various concentrations of M. SssI MTase in 0.1 M PBS (pH 7.4) containing 0.1 M AA. MTase concentrations (from left to right): 0.1, 0.5, 1, 5, 10, and 50 unit/mL. (B) Calibration curve. (C) Time-based photocurrent responses of the PEC biosensor after treatment with 1 unit/mL M. SssI MTase in 0.1 M PBS (pH 7.4) containing 0.1 M AA with light on and off. (D) Photocurrent response of the PEC biosensor after M. SssI MTase was inhibited with different concentrations of 5-fluorouracil. a–f: 0, 10, 50, 100, 150 and 200 μM.

concentrations of M. SssI MTase. Clearly, with increasing concentration of M. SssI MTase, the produced photocurrent at MBD1/dsDNA/AuNPs/BiOI/ITO decreased successively. As seen in Fig. 2(B), the photocurrent was proportional to the logarithm of M. SssI MTase concentration in the range from 0.1 to 50 unit/mL with the linear regression equation of $I(\mu A)=0.36\log c$ (unit/mL) − 1.34 ($R=0.9949$). The detection limit was estimated to be 0.035 unit/mL ($S/N=3$). The detection limit obtained was lower than that in some previous reports, such as 0.04 unit/mL (Yin et al., 2013b), 0.045 unit/mL (Yin et al., 2013a), 0.05 unit/mL (Li et al., 2012), 0.07 unit/mL (Su et al., 2012), 0.1 unit/mL (Wang et al., 2012), 0.12 unit/mL (He et al., 2011), 0.18 unit/mL (Wang et al., 2013), 0.3 unit/mL (Liu et al., 2009), 0.4 unit/mL (Zhao et al., 2013), and 0.8 unit/mL (Li et al., 2007).

The stability of the photocurrent responses of the fabricated biosensor was investigated (Fig. 2(C)). No significant change of photocurrent response was observed with light on and off which was repeated 14 times with the interval of 10 s (RSD = 2.0%), indicating a good stability. The reproducibility of the PEC biosensor was evaluated by intra- and inter-assay coefficients of variation (CV). M. SssI MTase with concentration of 1 unit/mL was determined repeatedly using the proposed biosensor 10 times; intra-assay CV was 7.58%. The inter-assay CV on five biosensors was 6.47%. Considering the low detection concentration of MTase, we think that the reproducibility of the PEC biosensor was satisfactory. The fabricated biosensor also presented good selectivity when using Dam, M. TaqI, M. EcoRI, M. BamHI, M. HhaI and M. HaeIII MTase as interferents (see Supporting information).

3.4. *Inhibition of M. SssI MTase activity*

DNA MTase plays an important role during carcinogenesis. Thus the pharmacological inhibitors for DNA MTase have promising applications in anticancer therapeutics. To further demonstrate the potential application of this PEC biosensor in screening DNA MTase inhibitors, the influence of 5-fluorouracil on M. SssI MTase activity was investigated. As shown in Fig. 2(D), the photocurrent increased with increasing concentration of 5-fluorouracil. This indicated that the activity of M. SssI MTase was inhibited and the methylation level of dsDNA on electrode surface was decreased, which resulted in a decreased immobilization amount of MBD1 protein and an increased photocurrent. The IC50 value was calculated to be 108.2 μM. This result also showed that the PEC biosensor has the potential ability to screen the inhibitors to DNA MTase.

4. Conclusions

In summary, we developed a sensitive PEC biosensor for the detection of MTase activity based on BiOI as photoactive material and MBD1 protein as recognition unit for methylation site. This method does not need sophisticated, large, and expensive instrumentation. The detection limit was achieved down to 0.035 unit/mL. Furthermore, the fabricated PEC biosensor has potential applications in screening of DNA MTase inhibitors. This assay platform could be applied also to detect other DNA MTase activity through changing the dsDNA sequences.

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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.2013.09.065.

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