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A novel signal-on strategy for *M.SssI* methyltransfease activity analysis and inhibitor screening based on photoelectrochemical immunosensor

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ABSTRACT

In this work, a novel signal-on photoelectrochemical (PEC) immunosensor was fabricated for *M.SssI* methyltransfease (MTase) activity analysis and inhibitor screening based on an *in situ* electron donor producing strategy, where the anti-5-methylcytosine antibody was selected as DNA CpG methylation recognition unit, gold nanoparticle labeled streptavidin (SA-AuNPs) as signal amplification unit and alkaline phosphatase conjugated biotin (ALP-Biotin) as enzymatic unit. In the presence of *M.SssI* MTase, hairpin DNA1 containing the palindromic sequences of 5′-CCGG-3′ in its stem was methylated. After hybridization with biotin-conjugated DNA2, the stem-loop structure of the hairpin DNA1 was unfolded and the duplex strand DNA (dsDNA) was formed. Then, the dsDNA was captured on the surface of anti-5-methylcytosine antibody modified electrode through the specific immuno-reaction. Afterwards, SA-AuNPs and ALP-Biotin was further captured on the electrode surface through the specific reaction between biotin and streptavidin. Under the catalysis effect of ALP towards ascorbic acid 2-phosphate trisodium salt (AAP), ascorbic acid (AA) was *in situ* produced as electron donor and a strong PEC response was obtained. The fabricated biosensor showed high detection sensitivity with low detection limit of 0.33 unit/mL for *M.SssI* MTase. Furthermore, the inhibition research suggested that RG108 could inhibit the *M.SssI* MTase activity with the IC_{50} value of 152.54 nM.

1. Introduction

DNA methylation, a crucial epigenetic modification of the genome, plays a significant role in both prokaryotes and eukaryotes (Reik et al., 2001). Indeed, aberrant DNA methylation is responsible to various diseases such as cancers (Esteller and Herman, 2002; Momparler and Bovenzi, 2000). In normal cells, most CpG islands spanning the promoter regions are unmethylated, and their downstream genes are transcriptionally active. In contrast, when promoter CpG islands in cancer cells are methylated, their downstream genes such as tumor suppressor genes are consistently silenced (Frigola et al., 2006). It is well known that DNA methylation is caused by DNA methyltransfease (MTase), which is able to transfer a methyl group from the methyl group donor of S-adenosylmethionine (SAM) to the C5-positon of cytosine or the 6-amino group of adenine at specific base sequence (Hermann et al., 2004; Nomura and Barbas, 2007). Therefore, the DNA methylation level is always linked to MTase activity and it is important to analyze the activity of DNA MTase and screen the corresponding inhibitors.

There are many traditional methods for analyzing DNA MTase activity and screening inhibitor, which are focused on radioisotope labeled substrate (Bergerat et al., 1991), bisulfate treatment rolling circle amplification (Cao and Zhang, 2012), colorimetric approaches (Song et al., 2009), PCR (polymerase chain reaction) (Lyko et al., 2000), HPLC (high-performance liquid chromatography) (Lopez Torres et al., 2011), SERS (surface enhanced Raman spectroscopy) (Hu and Zhang, 2012), fluorescence (Chen and Zhao, 2013), electrochemical method (Deng et al., 2014) and so on. However, all of them have limitations, for example, radioisotope labeled substrate are harmful to biological tissue, bisulfate treatment rolling circle amplification need to convert cytosine to uracil which is time-consuming. Although colorimetric methods are simple, gold nanoparticles are easy to aggregate. HPLC need sophisticated large-scale instrument, and PCR need a complex process. Thus, it is still necessary to develop rapid and simple methods for accurately detecting DNA methylation level and analyzing MTase activity.

Photoelectrochemical (PEC) assay is a rapidly developed and promising analytical method that possesses the advantage of remarkable sensitivity, simple instrument and easy operation. Thus, it was widely used in various analytical field, such as nucleic acids analysis (Liang and Guo, 2006; Zhang et al., 2013), some small biomolecules detection (Sun et al., 2014; Wang et al., 2009),

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protein detection (Zhao et al., 2012), ion detection (Chamier et al., 2010; Huang et al., 2013) and so on. However, the PEC assay designed for the detection of DNA MTase activity was reported rarely (Shen et al., 2015; Zhou et al., 2014). In our previous report, a PEC immunoassay had been designed for detection of methylated DNA based Bi_2S_3 nanorods, methyl bonding domain protein and anti-his tag antibody (Yin et al., 2014). The result indicates that the PEC immunoassay has the potential for DNA MTase activity analysis. Anti-5-methylcytosine antibody has attracted great interesting for a wide range of applications on DNA methylation analysis. Up to now, there are many methods for detecting DNA methylation based on anti-5-methylcytosine antibody such as electrochemiluminescence (ECL) based immunoassay technique (Kurita et al., 2012), colorimetric method based on microspheres (Ge et al., 2012a). We have also reported an electrochemical immunoassay for DNA MTase activity analysis based on anti-5-methylcytosine antibody (Wang et al., 2012). To the best of our knowledge, however, there are no report with respect to signal-on strategy for DNA MTase activity analysis and inhibitor screening based on PEC immunosensor.

In this article, a novel signal-on PEC immunosensor was fabricated for *M.SssI* MTase activity analysis and inhibitor screening based on *in situ* generating ascorbic acid as electron donor. The assay strategy is shown in Scheme 1. A hairpin DNA1 contained the palindromic sequences of 5′-CCGG-3′ in its stem, and this sequence could be recognized and methylated by *M.SssI* MTase. After methylation reaction, the probe DNA1 was hybridized with biotin-conjugated complementary DNA2, which unfolded the stem-loop structure of probe DNA1 and the two methylated cytosines was located at the both terminals of the formed double-strand DNA (dsDNA). Subsequently, the dsDNA could be captured on anti-5-methylcytosine antibody modified ITO electrode surface through the immuno-reaction between methylated cytosine and anti-5-methylcytosine antibody. Afterwards, based on the specific reaction between biotin and streptavidin, immunogold labeled streptavidin (SA-AuNPs) and alkaline phosphatase conjugated biotin (ALP-Biotin) were further captured on the electrode surface successively. Under the catalytic effect of ALP towards ascorbic acid 2-phosphate trisodium salt (AAP), ascorbic acid (AA) was *in situ* produced as electron donor. The higher *M.SssI* MTase activity could lead to the enhanced ALP loading amount, which could improve the amount of produced AA and further increase the detection sensitivity. For decreasing the effect of the changing electrode surface properties on the detection sensitivity, the Bi_2S_3 modified ITO electrode (Bi_2S_3/ITO) was used as working electrode for PEC measurement. After the detection buffer was incubated with the fabricated immunosensor, the PEC response was recorded by Bi_2S_3/ITO. Based on the change of the PEC response, the activity of *M.SssI* MTase could be detected using this signal-on strategy.

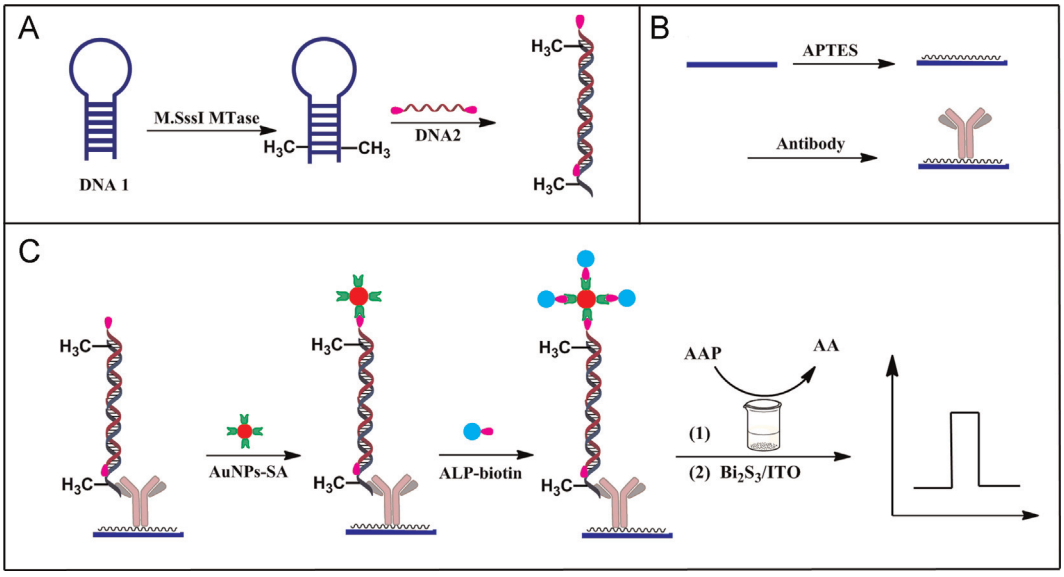
2. Experimental section

2.1. Reagents and apparatus

Anti-5-methylcytosine antibody was purchased from Abcam (Cambridge, UK). *M.SssI* MTase and Dam MTase were obtained from New England BioLabs (Ipswich, MA). N-phthalyl-L-tryptophan (RG108, the molecular structure was listed in Supplementary materials, Fig. S1) was purchased from Selleck (Houston, USA). Bismuth nitrate, sodium sulfide, carbamide, (hydroxymethyl)aminomethane (Tris), and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Aladdin (Shanghai, China). Biotin-ALP and streptavidin were supplied by Shanghai Sangon Biotechnology Co. (China). AAP was supplied by Shanghai Dibo Chemicals Co. Ltd. (China). Bovine serum albumin (BSA) was obtained from Sigma Aldrich (St. Louis, MO). Glutaraldehyde (GLD, 50% aqueous solution) was offered by Sinopharm Chemical Reagent Co., Ltd. (China). and purified by distillation before use. Indium tin oxide (ITO) was purchased from Zhuhai Kaivo Electronic Components Co., Ltd. (China, ITO coating 180 ± 25 nm, sheet resistance $< 15\ \Omega/cm^2$). AuNPs (Liu and Lu, 2006), SA-AuNPs (Li and Cui, 2013; Zhao et al., 2013) and Bi_2S_3 (Ge et al., 2012b) were synthesized according to the previous reports (see Supplementary materials).

The oligonucleotides used in this work were obtained from Shanghai Sangon Biotechnology Co. (China) and the base sequences are as follows. Hairpin DNA1: 5′-GGC CGT AGA GCT CCC TTC AAT CCA ACA TGA TAC GGC C-3′; DNA2: 5′-biotin-CAT GTT GGA TTG AAG GGA GCT CTA CGG CC-biotin-3′.

The stock buffer solutions used in this study are as follows. Anti-5-methylcytosine antibody dilution buffer, 0.01 M PBS and 30% glycerinum (pH 7.4). *M.SssI* stock buffer, 10 mM Tris–HCl, 50 mM NaCl, 1 mM DTT, 10 mM $MgCl_2$ (pH 7.4) and 30% glycerinum. DNA hybridization buffer, 10 mM Tris–HCl, 1.0 mM EDTA, and 1.0 M NaCl (pH 7.4). PEC detection buffer, 0.1 M Tris–HCl and



Scheme 1. Schematic illustration of the fabrication of PEC immunosensor and the detection of *M.SssI* MTase activity.

0.01 M AAP (pH 9.8). Blocking buffer, 0.01 M PBS (pH 7.4) containing 1% (w/v) BSA. Biotin-ALP dilution buffer, 0.01 M PBS and 30% glycerinum (pH 7.4). Electrode washing buffer, 10 mM Tris–HCl and 100 mM NaCl (pH 7.4). All aqueous solutions were prepared using double distilled water and stored at 4 °C before use.

Electrochemical impedance spectroscopy (EIS) was measured with a CHI660C electrochemical station (Austin, USA) with the three electrode system. Photoelectrochemical measure was performed by a CHI832A electrochemical station with a 500 W Xe lamp as the irradiation source.

2.2. Modifications of ITO electrode with anti-5-methylcytosine antibody

ITO slices were cleaned with acetone, NaOH (1 M, in ethanol and water with v/v=1:1) and distilled water, and dried at room temperature before use. Then silanized ITO was prepared according to a previous report (Nakanishi et al., 2010). In a typical experiment, the cleaned ITO electrode was immersed in ethanol solution containing 1% 3-aminopropyl triethoxysilane (APTES) for 30 min at 60 °C and rinsed three times by ethanol and water in turn (the electrode was noted as APTES/ITO). After activated by GLD, 20 μL of anti-5-methylcytosine antibody (10 μg/mL) was dropped on the APTES/ITO electrode and incubated for 2 h at room temperature in a humid cell. Then, the modified electrode was rinsed three times with washing buffer, and the nonspecific binding sites were blocked by blocking buffer for 30 min. The obtained electrode was noted as Antibody/APTES/ITO.

2.3. Methylation of hairpin DNA1

20 μL of 1×10^{-6} M hairpin DNA1 and 15 μL of 10 mM Tris–HCl buffer (pH=7.0) containing 1.28 mM SAM, 400 mM NaCl, 80 mM $MgCl_2$, and 8 mM dithiothreitol (DTT) were mixed firstly. Then, 5 μL of various concentrations of *M.SssI* MTase (final concentration was 1–50 unit/mL) were added into the above solution and incubated at 37 °C for 2 h. After that, the solution was further incubated at 65 °C for 30 min to make the *M.SssI* MTase inactivation. Finally, 40 μL of 1.0×10^{-6} M of complementary DNA2 were added into the solution to hybridize with hairpin DNA1 at 37 °C for 2 h, obtaining double-strand DNA-conjugated biotin (Biotin-dsDNA).

2.4. PEC immunosensor fabrication and PEC detection

The PEC immunosensor was prepared as shown in Scheme 1. 20 μL of biotin-dsDNA was dropped on the Antibody/APTES/ITO surface and incubated at 37 °C for 2 h. Then the electrode was washed three times with washing buffer to obtain the DNA/Antibody/APTES/ITO electrode. After that, 20 μL of SA-AuNPs were dropped on the DNA/Antibody/APTES/ITO surface and incubated at 37 °C for 1 h. The obtained electrode was noted as SA-AuNPs/DNA/Antibody/APTES/ITO electrode. In the end, the electrode was further incubated with 20 μL of biotin-ALP for 1 h, and then washed three times with washing buffer. This electrode was named as ALP/SA-AuNPs/DNA/Antibody/APTES/ITO.

For PEC detection, 40 μL of 3 mg/mL Bi_2S_3 dispersion was dropped on the bare ITO electrode surface and dried under infrared light irradiation. The obtained Bi_2S_3/ITO was used as working electrode. Then, the ALP/SA-AuNPs/DNA/Antibody/APTES/ITO was immersed into 10 mL of 0.1 M Tris–HCl containing 10 mM AAP solution (pH=9.8) under magnetic stirring at 37 °C for 1 h. Finally, the PEC response of Bi_2S_3/ITO was recorded in the above solution.

2.5. The assay of inhibitor and selectivity for M.SssI MTase

The solution for inhibitor assay were fabricated by mixing 20 μL of 1×10^{-6} M hairpin DNA and 20 μL of 10 mM Tris–HCl buffer (pH 7.0) containing 1.28 mM SAM, 400 mM NaCl, 80 mM $MgCl_2$, 8 mM dithiothreitol (DTT), 40 unit/mL *M.SssI* MTase and various concentrations of inhibitor (RG108) to make the final concentration of RG108 ranged from 10 to 1000 nM and the final concentration of *M.SssI* MTase of 20 unit/mL. Then, the mixed solution was incubated at 37 °C for 2 h. After that, it was further incubated at 65 °C for 30 min to make the *M.SssI* inactivation. Then the solution was used for PEC immunosensor preparation as described in Section 2.5 and the PEC response was recorded using Bi_2S_3/ITO as working electrode. The inhibition percentage of RG108 was estimated by the following equation:

$$\text{Inhibition percentage}\ (\%) = [(I_1 - I_2)/(I_1 - I_0)] \times 100\% \quad (1)$$

where I_0 is the catalytic PEC current of 0 unit/mL *M.SssI* MTase, I_1 is the catalytic PEC current of 20 unit/mL *M.SssI* MTase, and I_2 is the catalytic PEC current after the different concentration RG108 inhibitions.

To further investigate the selectivity of the proposed method, another DNA MTase (Dam MTase) was selected as the control enzyme. The investigation of selectivity was performed with 20 unit/mL Dam MTase as same as the detection procedure of *M.SssI* MTase activity, with the exception of the methylation step was replaced by Dam MTase.

3. Results and discussion

3.1. Characterization of the PEC immunosensor

The stepwise modification process of the PEC immunosensor was characterized by electrochemical impedance spectroscopy (EIS) in 5 mM $Fe(CN)_6^{3-/4-}$(1:1) solution containing 0.1 M KCl. As shown in Fig. 1A, the electron transfer impedance (R_{et}) of bare ITO electrode was about 100 Ω (curve a). However, after ITO was silanized by APTES, the R_{et} value decreased obviously (curve b), which can be ascribed to the amino group of APTES, which promotes the diffusion of negative charged redox probe of $Fe(CN)_6^{3-/4-}$ to electrode surface. When anti-5-methylcytosine antibody were immobilized on the APTES/ITO electrode surface and then blocked by BSA, the R_{et} value (curve c) increased significantly due to the steric hindrance effect of antibody. Subsequently, the R_{et} value further increased when methylated dsDNA were captured on the electrode surface, which can be ascribed to the electrostatic repulsion between phosphate backbone of DNA and $Fe(CN)_6^{3-/4-}$. Afterwards, when SA-AuNPs and biotin-ALP were immobilized on the electrode successively, the R_{et} value (curves e and f) increased successively owing to the steric hindrance effect. These results indicated the successful immobilization of SA-AuNPs and biotin-ALP in turn. The cyclic voltammetry (CV) was also used for characterizing electrodes (see Supplementary materials, Fig. S2). The obtained results suggested the successful fabrication of the biosensor.

3.2. Feasibility investigation of the PEC immunosensor

To investigate the feasibility of the PEC assay, the photocurrent of Bi_2S_3/ITO was recorded in PEC detection buffer, where the detection buffer was incubated with different electrodes. As shown in Fig. 1B only a weak photocurrent (4.70 μA) was observed for Bi_2S_3/ITO in the blank detection buffer (curve a). After Antibody/APTES/ITO electrode was incubated with Biotin-dsDNA (hairpin

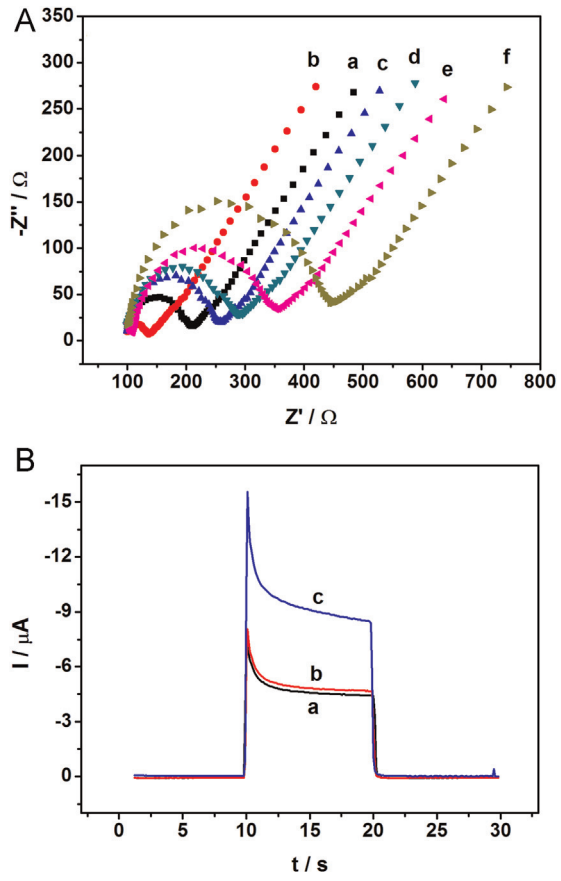


Fig. 1. (A) Complex plane impedance plots in 5 mM $Fe(CN)_6^{3-/4-}$(1:1) solution containing 0.1 M KCl at the bare ITO (a), APTES/ITO (b), antibody/APTES/ITO (c), DNA/antibody/APTES/ITO (d), SA-AuNPs/DNA/antibody/APTES/ITO (e) and ALP/SA-AuNPs/DNA/Antibody/APTES/ITO (f). (B) PEC responses of the Bi_2S_3/ITO in the detection buffer after it was incubated with different electrodes: (a) without incubation, (b) antibody/APTES/ITO incubated with Biotin-dsDNA (hairpin DNA was incubated with 0 U *M.SssI* MTase), SA-AuNPs and Biotin-ALP successively. (c) Antibody/APTES/ITO incubated with methylated Biotin-dsDNA, SA-AuNPs and Biotin-ALP successively. *M.SssI* MTase concentration was 10 unit/mL.

DNA was incubated with 0 U *M.SssI* MTase), SA-AuNPs and Biotin-ALP successively, and then the detection buffer was incubated with this electrode, the photocurrent was 4.91 μA (curve b), which was closed to the photocurrent obtained in the blank detection buffer, indicating that no catalytic factor was introduced into the detection buffer due to the absence of methylation process. However, the photocurrent increased significantly after the detection buffer was incubated with ALP/SA-AuNPs/DNA/Antibody/APTES/ITO, which clearly demonstrated that the Biotin-ALP immobilization could be triggered by the methylation event. According to the comparison of the photocurrent, we can conclude that the developed method could be applied to analyze the DNA MTase activity.

3.3. Effect of methylation time on the PEC response

In order to evaluate the effects of methylation time on the methylation process, the hairpin DNA1 was firstly incubated with *M.SssI* MTase for different time (ranged from 0 to 180 min), and then the biosensor was fabricated as described in Section 2.4. Finally, the PEC response of the Bi_2S_3/ITO was recorded in detection buffer after it was incubated with the biosensor for 1 h at 37 °C. As shown in Fig. 2, the PEC response increased obviously with extending the methylation time from 0 to 120 min, which indicated that more hairpin DNA1 was methylated with increasing the

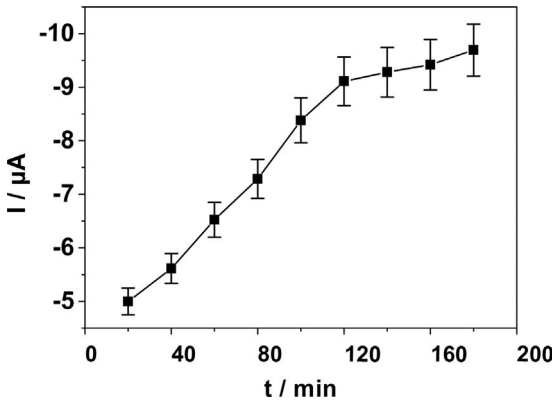


Fig. 2. Effect of methylation time on the PEC response.

methylation time in the selected time range. However, the PEC response increased slowly when further prolonging the methylation time to 180 min, indicating the rate of the *M.SssI* catalyzed methylation reaction was reduced. This phenomenon is may be attributed to the approximate saturation of the methylation level and the consumption of SAM. Therefore, 120 min was selected as optimization of methylation time.

3.4. The activity of M.SssI MTase detection

To further validate the ability of the PEC immunosensor for sensitively quantifying analysis of *M.SssI* MTase activity, the hairpin DNA1 was firstly incubated with different concentrations of *M. SssI* MTase, and then ALP/SA-AuNPs/DNA/Antibody/APTES/ITO was fabricated with the process as described in Section 2.4. Afterwards, the detection buffer was incubated with the fabricated biosensors and the photocurrent of Bi_2S_3/ITO was recorded. As shown in Fig. 3A, the photocurrent increased gradually with the increase of *M.SssI* MTase concentration from 1 to 50 unit/mL. With increasing the concentration of *M.SssI* MTase, the methylation level increased accordingly, which led to the increased capture amount of Biotin-ALP. As a result, the hydrolysis amount of AAP and the produced electron donor of AA were also increased. The high concentration of *M. SssI* MTase on the effect of the biosensor response was also investigated. The results indicated that when the concentration of *M. SssI* MTase was higher than 90 unit/mL, the PEC response achieved to level off, indicating the saturation of methylation reaction (see Supplementary materials, Fig. S3). The photocurrent showed a linear relationship with the concentration of *M.SssI* MTase in the range of 1–50 unit/mL (Fig. 4B). The linear regression equation could be expressed as $I_{pc} = -0.08392c - 8.22365$ (μA, unit/mL, $R = 0.9945$) and the detection limit was estimated to be 0.33 unit/mL (S/N = 3), which is lower than some of previous reports (Table 1).

For a kind of new detection method, the detection specificity is an important parameter. Therefore, in order to evaluate the specificity of the proposed method, Dam MTase was selected as the control enzyme, which can selectively methylate the adenine residues in the double strand DNA symmetric 5′-GATC-3′ sequence. As shown in Fig. 3C, the photocurrent for 20 unit/mL of *Dam* MTase was lower than that for 20 unit/mL of *M.SssI* MTase and close to that for 0 unit/mL, indicating that *Dam* MTase could not methylate the cytosine in stem of the hairpin DNA1 with the sequence of 5′-CCGG-3′, which resulted in the failure in capturing the Biotin-ALP on the electrode surface. This result also demonstrated the good detection specificity of the developed method.

The stability is another crucial factor for the proposed assay method, and it is evaluated by measuring the photocurrent of Bi_2S_3/ITO in detection buffer. As shown in Fig. 3D, there is no

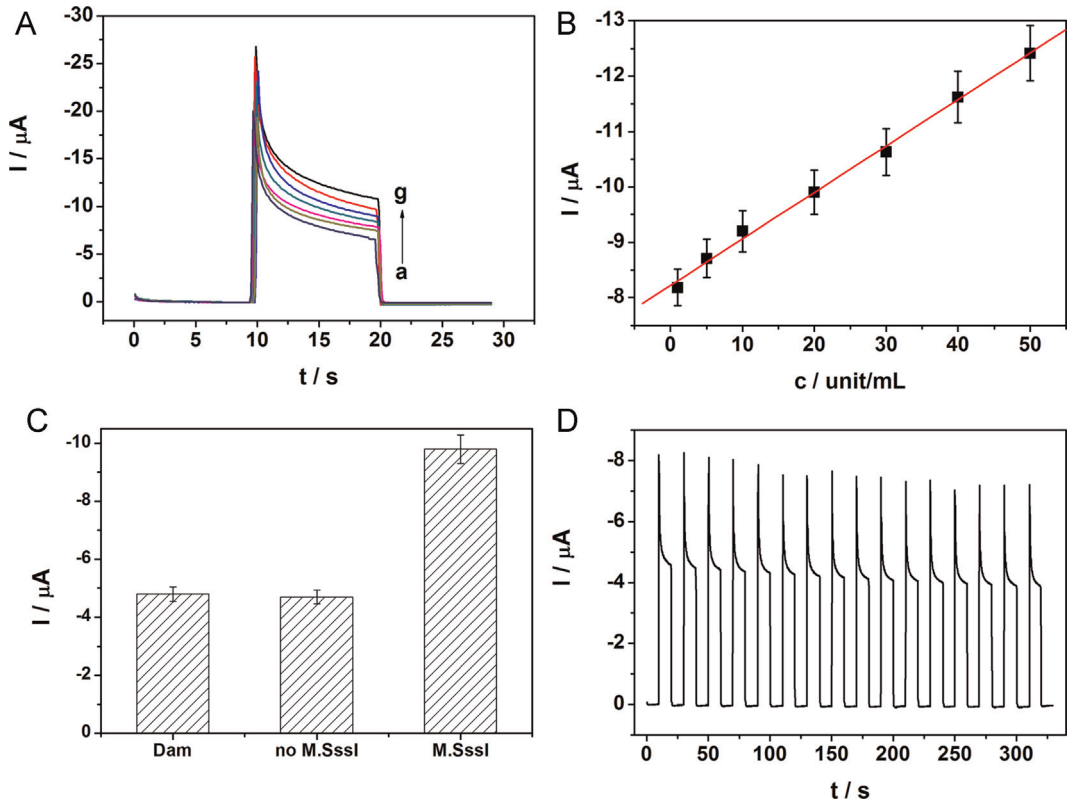


Fig. 3. (A) PEC response of Bi_2S_3/ITO in detection buffer after it was incubated with ALP/SA-AuNPs/DNA/Antibody/APTES/ITO, where the hairpin DNA1 was methylated with different concentrations of *M.SssI* MTase. (a–g) 1, 5, 10, 20, 30, 40, and 50 unit/mL; (B) the calibration curve of the *M.SssI* MTase activity assay; (C) the histograms for PEC response of Bi_2S_3/ITO in detection buffer after the detection buffer was incubated with ALP/SA-AuNPs/DNA/Antibody/APTES/ITO, where the hairpin DNA1 was incubated with different DNA MTase: (a) 20 unit/mL Dam MTase, (b) 0 unit/mL *M.SssI* MTase, and (c) 20 unit/mL *M.SssI* MTase. (D) PEC response of the Bi_2S_3/ITO in detection buffer for 16 times.

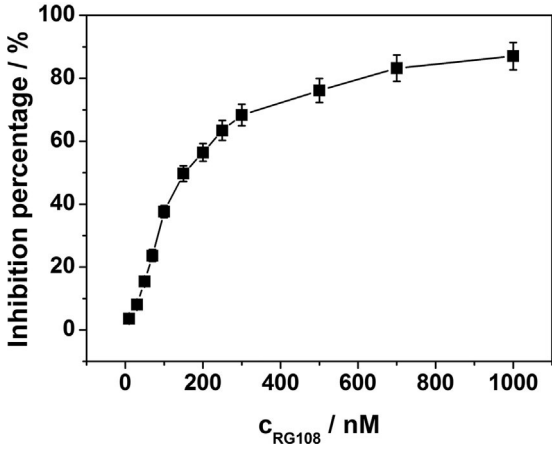


Fig. 4. The inhibition effect of RG108 on *M.SssI* MTase activity.

noticeable change of photocurrent response observed with light on and off continuing 320 s for 16 times. And the relative standard deviation (RSD) was 4.59% indicating the Bi_2S_3/ITO has good stability.

The reproducibility of the PEC immunosensor was also assessed by measuring the photocurrent of Bi_2S_3/ITO in detection buffer after it was incubated with ALP/SA/DNA/Antibody/APTES/ITO electrode (DNA was methylated by 20 unit/mL *M.SssI* MTase) for 1 h at 37 °C. The RSD for six independently measurements (six electrodes prepared dependently and six detection buffers) was 4.65%. These results indicate that the PEC immunosensor has an acceptable reproducibility.

Table 1
Comparison of detection performances of the developed method with other methods for DNA MTase.

Method	Linear range (unit/mL)	Detection limit (unit/mL)	References
Colorimetry	6–100	6	Li et al. (2010)
Colorimetry	2.5–40	2.5	Song et al. (2009)
Fluorescent	0.5–100	0.1	Ouyang et al. (2012)
Electroluminescence	0.05–100	0.02	Li et al. (2012)
Electrochemistry	0.25–10	0.18	Wang et al. (2013)
Photoelectrochemistry	1–50	0.33	This work

3.5. The inhibition of RG180 on the activity of M.SssI

Since DNA methylation plays crucial roles in both prokaryotes and eukaryotes, and aberrant DNA methylation levels have related to some cancers, it is important to discovery valid pharmaceuticals with inhibition activity on DNA MTase. Therefore, to further testifies the application ability of the developed method for DNA MTase inhibitor screening, the influence of RG108 (a kind of DNA MTase inhibitor) on *M.SssI* MTase activity was investigated. RG108 is a novel small molecule that effectively blocked DNA methyltransferases in vitro and did not cause covalent enzyme trapping in human cell lines (Brueckner et al., 2005). As shown in Fig. 4, the inhibition percentage increased with increasing RG108 concentration, and the maximum inhibition was achieved to 87.02%. From Fig. 4, the IC_{50} value of RG108 for *M.SssI* MTase was calculated to be 152.54 nM, which was closed to prior report of

115.2 nM (Brueckner et al., 2005), and the small distinction may be caused by different detection methods. These results demonstrated that the proposed method presented potential in analyzing the inhibition effects of anticancer drugs on DNA MTase activity and screening DNA MTase inhibitors.

4. Conclusion

In conclusion, we successfully fabricated a signal-on PEC immunosensor for *M.SssI* MTase activity analysis and inhibitor screening based on *in situ* generating AA as electron donor. With the assistance of the amplification units of SA-AuNPs and Biotin-ALP, the PEC immunosensor presented acceptable detection sensitivity with detection limit of 0.33 unit/mL. The developed method provides simple and rapid analysis because it is free from bisulfite treatment, PCR amplification, DNA digestion, or separation using chromatography techniques. In addition, it could be easily extended to screen its suitable inhibitors, which might be a help for the discovery of anticancer 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.2014.11.015.

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