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A superstructure-based electrochemical assay for signal-amplified detection of DNA methyltransferase activity

Hui Zhang [a,b,*], Yin Yang [a,b], Huilei Dong [a,b], Chenxin Cai [a,b,*]

[a] Jiangsu Key Laboratory of Biomedical Materials, Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, National and Local Joint Engineering Research Center of Biomedical Functional Materials, Nanjing Normal University, Nanjing 210097, PR China
[b] Jiangsu Key Laboratory of New Power Batteries, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210097, PR China

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ABSTRACT

DNA methyltransferase (MTase) activity is highly correlated with the occurrence and development of cancer. This work reports a superstructure-based electrochemical assay for signal-amplified detection of DNA MTase activity using M.SssI as an example. First, low-density coverage of DNA duplexes on the surface of the gold electrode was achieved by immobilized mercaptohexanol, followed by immobilization of DNA duplexes. The duplex can be cleaved by BstUI endonuclease in the absence of DNA superstructures. However, the cleavage is blocked after the DNA is methylated by M.SssI. The DNA superstructures are formed with the addition of helper DNA. By using an electroactive complex, RuHex, which can bind to DNA double strands, the activity of M.SssI can be quantitatively detected by differential pulse voltammetry. Due to the high site-specific cleavage by BstUI and signal amplification by the DNA superstructure, the biosensor can achieve ultrasensitive detection of DNA MTase activity down to 0.025 U/mL. The method can be used for evaluation and screening of the inhibitors of MTase, and thus has potential in the discovery of methylation-related anticancer drugs.

1. Introduction

DNA methylation, mainly involving addition of a methyl group at carbon 5′ (C5) of the pyrimidine ring in cytosine residues to give 5-methylcytosine (5-mC) (Stains et al., 2006; Branciamore et al., 2010), is one of the most important research areas on epigenetic modification of genomic DNA. According to numerous studies, aberrant methylation may cause changes in the DNA structure and in the stability and interaction mode between DNA and proteins, thus affecting gene expression. This may lead to many neurodegenerative diseases, immune system diseases and even cancer (Szyf, 2003; Skiriute et al., 2013). For example, the abnormally high methylation of *RASSF1A* gene CpG island has been confirmed in a wide variety of tumors, such as bladder cancer, rectal cancer and lung cancer (Yu et al., 2002).

In mammals, abnormal methylation is caused by DNA methyltransferase (MTase), which catalyzes the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to the C5-position of cytosine (Wu and Morris, 2001). Therefore, DNA methylation level is closely associated with the activity of DNA MTase. Notably, DNA MTases have become predictive biomarkers and potential therapeutic targets in variety of cancer types (Issa et al., 1993; Mutze et al., 2011; Belinsky et al., 1996; Kobayashi et al., 2011). Moreover, because abnormalities in MTase activity usually occur well in advance of other signs of malignancy, they could be used for early cancer diagnosis (Baylin and Herman, 2000; Das and Singal, 2004). Thus, the development of sensitive activity assays and inhibitor (anti-methylation drugs) screening for MTase is highly desirable in both clinical diagnostics and therapeutics. Up to now, a variety of biochemical assays has been developed for MTase activity assay, involving polymerase chain reaction (Yang and Zheng, 2014), fluorescence (Ji et al., 2014; Gao et al., 2014; Xing et al., 2014), surface plasmon resonance (Carrascosa et al., 2014), colorimetry (Wu et al., 2013), capillary electrophoresis (Fraga et al., 2002), high performance liquid chromatography (HPLC) (Torres et al., 2011), liquid chromatography/mass spectrometry (Salyan et al., 2007), chemiluminescence (Zeng et al., 2013), electrochemiluminescence (Zhao et al., 2015; Li et al., 2012a, 2012b), photoelectrochemistry (Shen et al., 2015; Zhou et al., 2014) and electrochemistry (Deng et al., 2014; Furst et al., 2014; Li et al., 2012a, 2012b, 2015; Liu et al., 2011; Muren and Barton, 2013; Su et al., 2012; Wang et al., 2015, 2012a, 2012b, 2012c, 2013; Wu et al., 2012; Zhang et al., 2015; Zhou et al., 2015). Among these methods, electrochemical techniques offer attractive advantages of low-cost, portability, simple

* Corresponding authors at: Jiangsu Key Laboratory of Biomedical Materials, National and Local Joint Engineering Research Center of Biomedical Functional Materials, Jiangsu Key Laboratory of New Power Batteries, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210097, PR China.
E-mail addresses: zhangh@njnu.edu.cn (H. Zhang), cxcai@njnu.edu.cn (C. Cai).

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operation, and rapid response.

Traditional electrochemical methods for MTase activity assay have frequently employed labeling with electroactive molecules and detection using restriction enzymes. Typically, DNA hybrids immobilized on the surface of the electrode are labeled with electroactive molecules, such as ferrocene acetic acid (Liu et al., 2011) and methyl blue (Muren and Barton, 2013; Su et al., 2012). After methylation of the electroactive labeled DNA hybrids, the electrodes are treated with a restriction enzyme which is sensitive to methylation at this site. When the DNA is fully methylated by active MTase, the restriction enzyme cannot cleave the DNA, and the electrochemical signal of the labels is preserved. In contrast, when the DNA is not completely methylated by MTase, the restriction enzyme can cleave the unmethylated DNA, resulting in a significant decrease in the number of electroactive labels and corresponding signal loss. The decrease in voltammetric signal after cleavage is related to the DNA methylation status and MTase activity, thereby forming the basis of the MTase activity assay. In order to amplify the electrochemical response and improve the sensitivity, signal amplification methods are combined with this assay. For example, thionine/graphene oxide (Li et al., 2012a, 2012b) and enzyme (such as AuNPs–IgG–HRP) (Wang et al., 2012a, 2012b, 2012c) composites have been employed to generate an amplified electrochemical signal. However, despite some improved performance, tedious labeling procedures and multistep processes are involved in these assays.

Since the first reported DNA detection by the Plaxco group (Xia et al., 2010), the DNA superstructure has been widely used as an efficient signal amplification in the measurement of DNA, proteins, cells, and metal ions (Chen et al., 2013; Wang et al., 2012a, 2012b, 2012c, Chen et al., 2012; Yuan et al., 2011; Liu et al., 2013). The DNA superstructure consists of a long DNA concatemer hybridized from two single-stranded DNAs that have partially complementary segments, which can effectively amplify the signal. However, the superstructure assay is also limited since numerous single-stranded DNAs participate in the construction of the DNA concatemer. Performance is impeded by the crowding (steric) effect, probe entanglement, and nonspecific interactions between probes. Recent studies show that the efficiency of signal amplification, sensitivity and reproducibility of the DNA superstructure assay are closely related to the density of DNA recognition probes, with low probe density favoring superior biosensor performance (Ge et al., 2014; Yang et al., 2014).

In this paper, we developed a DNA superstructure-based electrochemical assay for signal-amplified detection of DNA MTase activity. For the target DNA (DNA2; see Supplementary information S1.1 for sequences), we chose the 32-mer sequence from the promoter region of the *Homo sapiens RASSF1* gene, which is a candidate tumor suppressor gene (Dammann et al., 2000). Loss of expression is correlated with methylation of the CpG-island promoter sequence of *RASSF1A*. In order to control the density of DNA probes, mercaptohexanol (MCH) was first immobilized on the surface of the gold electrode. The electrode was then modified with a low density of thiolated double-stranded DNA (ds-DNA). The DNA superstructure was then constructed via helper DNA (DNA3 and DNA4) hybridization. An electrochemical reporter ($[Ru(NH_3)_6]^{3+}$, RuHex) was used as the signaling molecule due to its ability to bind to the anionic phosphate backbone of DNA strands via electrostatic forces (Chen et al., 2012). The large number of reporters carried by the DNA superstructure could achieve ultrasensitive detection of DNA MTase activity down to 0.025 U/mL. In addition, we demonstrated the application of our method for MTase evaluation and inhibitor screening, which will be helpful in the discovery of methylation-related drugs.

2. Experimental

2.1. Materials and reagents

MCH was purchased from Sigma-Aldrich (St. Louis, MO, USA). Tris (hydroxymethyl) aminomethane (Tris) and tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 98%) were purchased from Aladdin (Shanghai, China). Hexaammineruthenium(III) chloride ($[Ru(NH_3)_6]^{3+}$, RuHex) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 5-Aza-2′-deoxycytidine (5-Aza-dC), 5-azacytidine (5-Aza) and 2′,3′-dideoxy-3′-thiacytidine (Lamivudine, 3-TC) were purchased from TCI (Shanghai) Development Co., Ltd. S-Adenosyl-L-methionine (SAM), *E. coli* CpG MTase M.SssI and restriction endonuclease BstUI were supplied by New England BioLabs (Ipswich, MA). All other chemicals were of analytical reagent grade.

The HPLC-purified DNA was purchased from Shanghai Sangon Biological Engineering Technology and Services Co. Ltd. (Shanghai, China). The sequences are listed in the Supplementary information section S1.1.

2.2. DNA self-assembly on gold electrode

A 20 μL aliquot of ds-DNA (5.0 μM) (ds-DNA preparation is described in S1.2.) was freshly reacted with 10 μL of TCEP solution (10 mM) for 1 h to reduce the disulfide bond at the 3′-terminus of ds-DNA and generate a free thiol group for surface immobilization, followed by diluting the mixture to 100 μL with Tris-HCl buffer. For heterogeneous monolayer preparation, the cleaned gold electrode was immersed in 5 μM MCH solution for the proper time at room temperature, followed by incubation in ds-DNA solution for 16 h at 4 °C. Then, the electrode was rinsed thoroughly with Tris-HCl buffer.

2.3. Methylation of CpG and inhibition

The methylation of ds-DNA was performed at 37 °C for 2 h in 10 mM Tris-HCl buffer (pH 7.9) containing 160 μM SAM, 50 mM NaCl, 1 mM EDTA, and various concentrations of M.SssI (from 0 to 200 U/mL).

2.4. Cleavage by BstUI endonuclease

BstUI cleavage was performed at 37 °C in 10 mM Tris–HCl buffer (pH 8.5) containing 10 mM $MgCl_2$, 100 mM KCl, 0.1 mg/mL BSA and 20 U/mL BstUI at 37 °C for 2 h. After cleavage, the electrode was thoroughly washed with Tris-HCl buffer.

2.5. Superstructure DNA Hybridization

Hybridization was conducted at 37 °C by incubating the ds-DNA assembled electrode in 100 μL of Tris-HCl buffer containing 2 μM of each helper (DNA3 and DNA4) for 3 h. After hybridization, the electrode was thoroughly rinsed with Tris-HCl buffer.

2.6. Apparatus

Electrochemical impedance spectroscopy (EIS) measurements were performed on an Autolab PGSTAT-30 potentiostat/galvanostat (Eco Chemie BV, Utrecht, The Netherlands). Chronocoulometry (CC) and differential pulse voltammetry (DPV) were performed on a CHI 760C electrochemical workstation (Chenhua, Shanghai, China). All experiments were carried out with a conventional three-electrode system, with the modified gold as the working electrode and a Pt wire and a saturated calomel electrode (SCE) as the counter and reference electrodes, respectively. All DPV datas

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were recorded in a 10 mM Tris–HCl buffer (pH 7.4). Before DPV measurement, the electrodes were dipped in 50 μM RuHex for 1 min, removed, rinsed with distilled water and then dried with nitrogen.

3. Results and discussion

3.1. Detection principle of DNA MTase activity assay based on superstructure

The strategy for DNA MTase activity detection based on site-specific cleavage by the endonuclease and signal amplification of the DNA superstructure is presented in Scheme 1. The sequence DNA1 is complementary to part of the DNA2. During the hybridization, DNA2 partially binds to DNA1 to form ds-DNA, which contains the specific sequence recognized by BstUI endonuclease (from a Bacillus stearothermophilus U458, recognizing the sequence 5′-CG↓CG-3′). The remaining sequence of DNA2 is complementary to one terminus of helper DNA3; the other terminus of DNA3 hybridizes with one terminus of the helper DNA4. Thus, a long DNA superstructure containing numerous alternating DNA3 and DNA4 probes is formed. As shown in Scheme 1, to achieve optimal low probe density, we first immobilize MCH on the gold electrode surface. Then the DNA1/DNA2 duplexes are immobilized on the gold electrode by forming Au–S bonds. After the duplex is methylated by M.SssI, the cleavage by BstUI is blocked, which allows superstructure formation in the presence of helper DNA3&DNA4. The modified electrode is immersed in a solution containing RuHex, Numerous RuHex can be gathered on the working electrode via DNA nanostructures, eventually resulting in significantly amplified electrochemical signals. However, when the DNA1/DNA2 duplex is not methylated, the ds-DNA was cleaved by restriction enzyme BstUI, no DNA superstructure can form, and the electrochemical signal cannot be enhanced. Thus, the amplified electrochemical signal is related to the MTase activity and establishes the basis of the MTase activity assay. In short, the site-specific cleavage by BstUI (resulting a high selectivity) and the extraordinary signal amplification of the DNA superstructure (resulting in a high signal-to-background ratio) enable a highly sensitive and selective assay for MTase activity.

3.2. Feasibility of the strategy

To investigate the feasibility of the proposed strategy for MTase activity detection, some control experiments are performed. As shown in Fig. 1, the DPV response of the RuHex shows a reduction peak at approximately −225 mV (curve a), which corresponds to

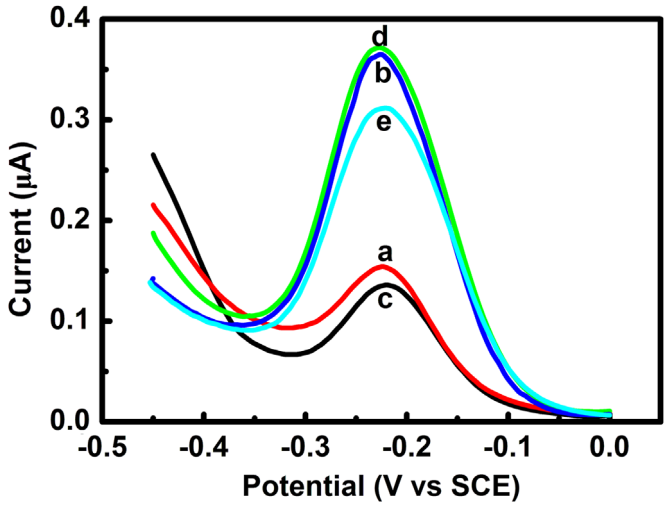
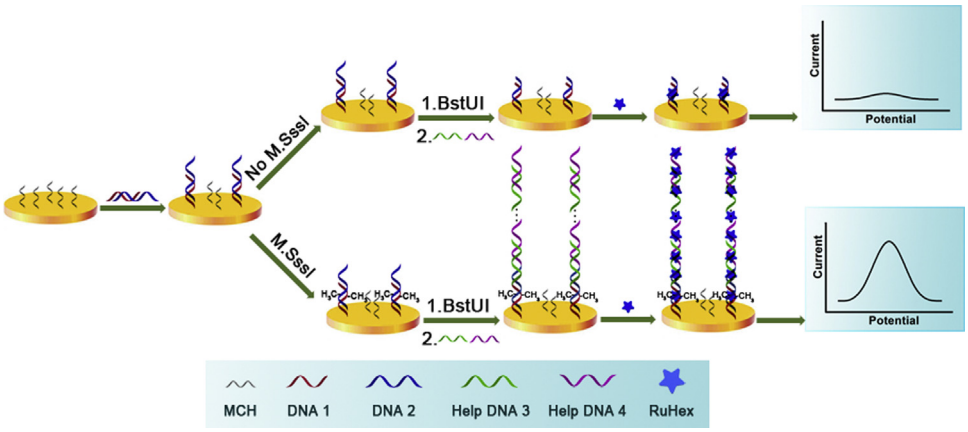


Fig. 1. DPV responses of the (a) MCH/ds-DNA modified gold electrode; MCH/ds-DNA modified gold electrode treated with (b) DNA3&DNA4, (c) BstUI (20 U/mL) and DNA3&DNA4, (d) M.SssI (160 U/mL), SAM (160 μM), BstUI (20 U/mL) and DNA3&DNA4 and (e) M.SssI (80 U/mL), SAM (160 μM), BstUI (20 U/mL) and DNA3&DNA4.

the reductive process of the adsorbed electroactive RuHex on the DNA negatively charged backbone (Steichen et al., 2007). After treatment of the MCH/ds-DNA modified gold electrode with DNA3&DNA4, the peak current of RuHex sharply increased (curve b), because the longer DNA superstructure formed, and more RuHex adsorbed on the DNA backbone. When the MCH/ds-DNA modified GCE was treated with BstUI and DNA3&DNA4 (curve c), the peak current of RuHex was smaller than that of curve a, because ds-DNA was cleaved by BstUI, and the DNA superstructure could not form. But if the MCH/ds-DNA modified GCE was treated with MSssI, SAM and then BstUI and DNA3&DNA4, the DPV value of the RuHex was the same as that for curve b (curve d), because after the ds-DNA was methylated by M.SssI, the cleavage site of the endonuclease was blocked by CpG methylation, and BstUI endonuclease could not cleave the ds-DNA. If we decreased the amount of M.SssI, the ds-DNA could not be fully methylated, and only part of ds-DNA was cleaved by BstUI, resulting in a decrease in the DPV signal (curve e). The above results show that the electrochemical signal of RuHex is related to the MTase activity and can be used to detect the MTase activity.

3.3. Optimization of MCH immobilization time and methylation time

A low density of DNA1/DNA2 duplexes is important for



Scheme 1. Schematic illustration for detecting MTase activity based on electrochemical signal amplification by DNA superstructures in the low-density approach.

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superstructure formation. In order to determine the optimum conditions, we used chronocoulometry (CC) to measure the amount of adsorbed RuHex on the DNA backbone, thus reflecting the number of DNA strands on the electrode surface. According to the Cottrell equation,

$$Q=\frac{2nFAD_0^{1/2}C_0^{*}}{\pi^{1/2}}t^{1/2}+Q_{dl}+nFA\Gamma_0 \quad (1)$$

where Q_{dl} is the capacitive charge (C) and $nFA\Gamma_0$ is the charge of RuHex adsorbed on the DNA backbone. In the absence of RuHex, the CC intercept at t=0 is the Q_{dl} when there is no adsorbed RuHex, and in the presence of RuHex, the intercept is the sum of Q_{dl} and $nFA\Gamma_0$. Therefore, the amount of adsorbed RuHex can be determined from the difference in CC intercepts in the presence and absence of RuHex. As shown in Fig. S2A, for the MCH modified electrode, the intercepts were nearly identical in the absence and presence of RuHex, indicating that there is negligible nonspecific adsorption of RuHex on the MCH-modified electrode (Steel et al., 1998). But for the MCH-ds-DNA modified electrode, the difference of intercepts was obvious, indicating some RuHex was adsorbed on the backbone of DNA. And for the MCH/ds-DNA/DNA3&DNA4 superstructure-modified electrode, the difference of intercepts increased greatly, indicating that more RuHex was adsorbed on the DNA backbone of the superstructure. Since the amount of MCH immobilized on the surface of electrode has a great influence on the density of ds-DNA, CC can be used to optimize the MCH coverage.

We fixed the MCH concentration at 5 μM, and then adjusted the MCH immobilization time. As shown in Fig. S2B, with increasing immobilization time, the $nFA\Gamma_0$ increased and reached a maximum with 5 min immobilization time. With longer immobilization time, the surface was covered by MCH and the density of ds-DNA decreased, leading to suppression of the subsequent DNA superstructure assembly. Therefore, the optimal immobilization time for MCH was 5 min

We compared our approach with traditional DNA modified-electrode approach (DNA immobilized on the electrode surface first, followed by immobilization of MCH to remove nonspecifically adsorbed DNA). In Fig. S3A (low-density approach) and B (traditional self-assembled approach), the red lines correspond to the DPV response of RuHex when the ds-DNA (unmethylated) was treated with BstUI endonuclease, and the black lines show the DPV response of RuHex when the ds-DNA (methylated) was treated with BstUI and helper DNA (DNA3&DNA4). The signal change was much larger for the low-density approach. By comparing the two approaches under the same experimental conditions, it can be concluded that the low-density approach can amplify the measured electrochemical signal and thus enhance the assay sensitivity.

To evaluate the effects of methylation time on the methylation process, the ds-DNA was methylated by M.SssI for different times, and the DPV signal was recorded after the methylated DNA was treated with BstUI for 2 h. As shown in Fig. S4, with an increase of methylation time, the DPV peak current increased progressively for 2 h and then reached a plateau, indicating the ds-DNA was fully methylated and BstUI could not cleave it. Therefore, 2 h was chosen as the optimal time for the M.SssI methylation time throughout the subsequent experiments.

3.4. Electrochemical analysis of DNA MTase activity

Under the optimal conditions, we applied this method to quantitatively determine the M.SssI activity. As shown in Fig. 2A, the DPV peak current increased with increasing concentration of M.SssI. Fig. 2B shows that the signal increases linearly with M.SssI concentration (in U/mL) from 0.5 to 120 U/mL. With a correlation coefficient (R) of 0.995, the regression equation could be expressed as

$$\Delta I(\mu A) = 0.84411 + 0.21327 \lg [C] \quad (2)$$

where ΔI is the increase in current and C is the concentration of M.SssI in U/mL. The detection range of the method is much wider than that of the colorimetric approach (1.0–10 U/mL) (Wu et al., 2013), a previously reported HPR-based electrochemical immunosensing method (0.5–50 U/mL) (Wang et al., 2012a, 2012b, 2012c), and a recently obtained electrochemical approach (0.5–60 U/mL) (Zhang et al., 2015) (see table S1). The detection limit was estimated as 0.025 U/mL at a signal/noise of 3, which is comparable with that recently obtained using the electrochemical approach (0.025 U/mL) (Zhou et al., 2015), and much lower than that of the electrochemical approach using polyaniline (0.12 U/mL) (Zhang et al., 2015), the colorimetric approach using cross-linking AuNPs aggregation (2.5 U/mL) (Song et al., 2009) and methylation-responsive DNAzyme methods (6 U/mL) (Li et al., 2010) (see table S1). A series of 10 repetitive measurements with 80 U/mL M.SssI was used for evaluating the precision of the proposed method, and a relative standard deviation (RSD) of ~3.5% was obtained, demonstrating good reproducibility of the assay. These results suggest that the method can be used for convenient and efficient analysis of MTase activity.

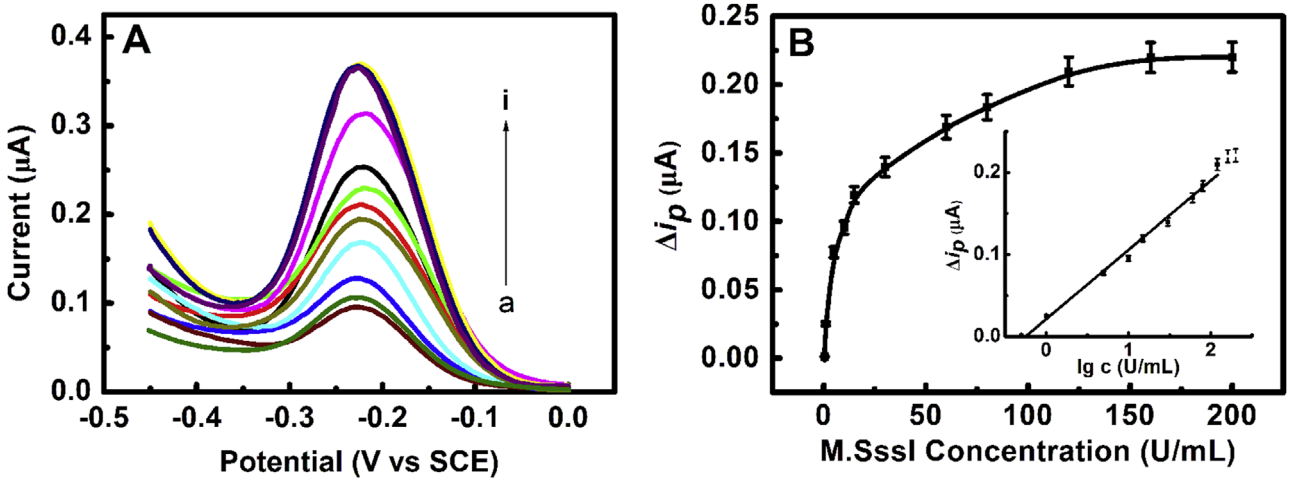

Fig. 2. (A) Effect of M.SssI concentration on the DPV response of the DNA superstructure. The concentration of M.SssI is (a) 0 (b) 0.5, (c) 1.0, (d) 5.0, (e) 10, (f) 15, (g) 30, (h) 60, (i) 80, (j) 120, (k) 160, and (l) 200 U/mL. (B) The plot of DPV signal increase vs M.SssI concentration. Inset: linear plot of Δi_p vs. lg c. Error bars: SD, n=3.

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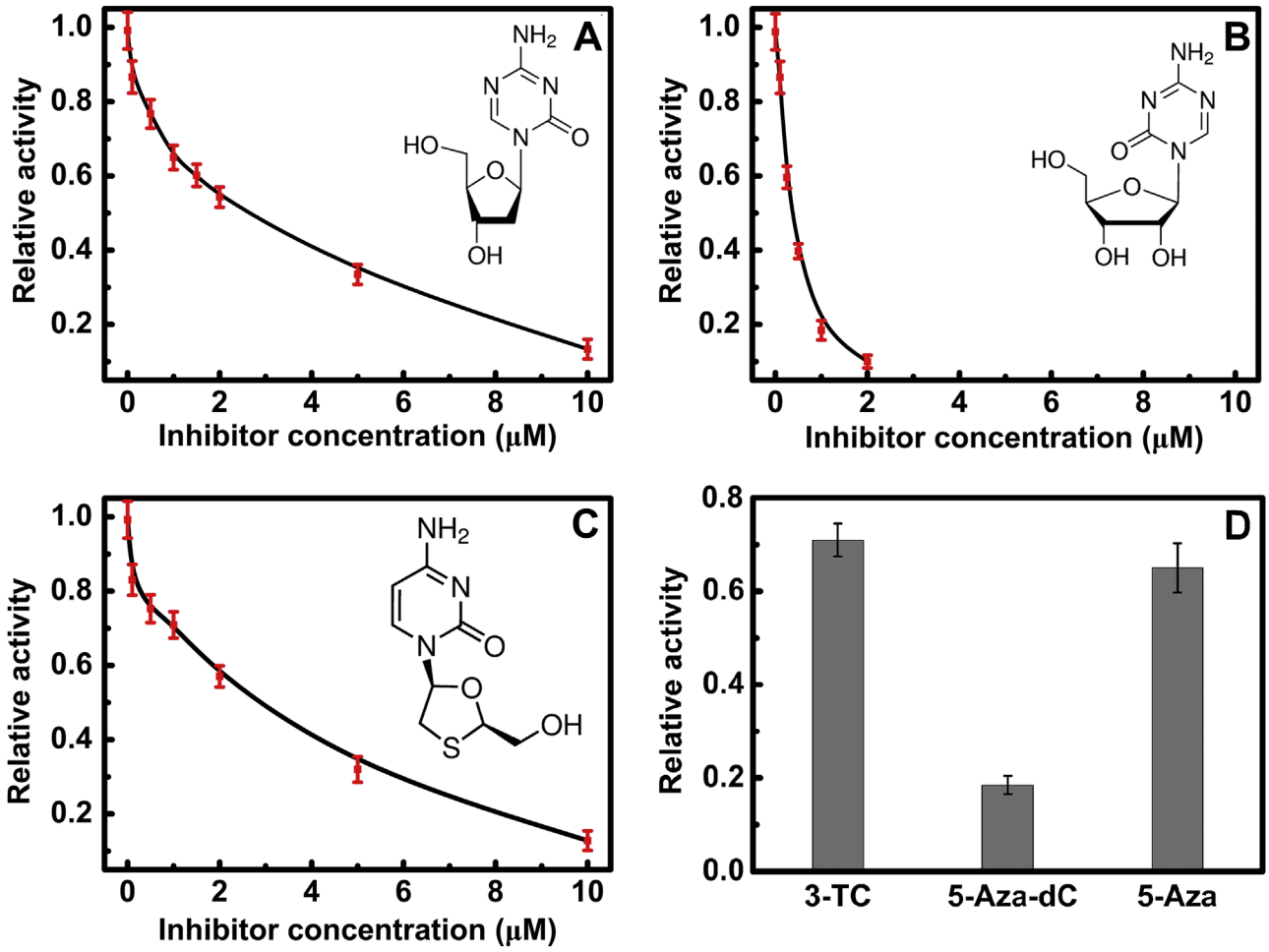

Fig. 3. Dependence of the current signal on the type of MTase. Error bars: SD, n=3.

3.5. *Selectivity of the proposed M.SssI activity assay*

The selectivity of the proposed M.SssI activity assay was evaluated by selecting two other cytosine methyltransferases (HaeIII and AluI) as the potential interfering MTase. HaeIII methylates the internal cytosine residues in the sequence of the symmetric tetranucleotide 5′-GGCC-3′, and AluI methylates the cytosine residues in the double-stranded symmetric 5′-AGCT-3′ sequence [Ji et al., 2014]. As shown in Fig. 3., the proposed assay displays high specificity to M.SssI. The analytical signal (ΔIp) of M.SssI is ~27 and 20 times higher than that of HaeIII and AluI, respectively. This is evidently due to the fact that the CpG dinucleotide site in the HpaII recognition sequence (5′-CCGG-3′) cannot be methylated by the HaeIII or AluI. Therefore, the proposed assay exhibits good selectivity toward M.SssI activity assay over HaeIII and AluI.

3.6. *Evaluation of MTase activity inhibition*

Many in vitro studies have indicated that MTase inhibitors have a reverse effect on the biological behavior of a variety of malignant solid tumors, by reduction of the high methylation level in DNA methylation-associated tumors and cancers. Therefore, the development of MTase inhibitors has provided an effective tool for epigenetic cancer therapy (Wei et al., 2014; Kang et al., 2014). We investigated the applicability of our method in evaluating and screening inhibitors of M.SssI using of 5-Aza, 5-Aza-dC, and 3-TC as model MTase inhibitors. These compounds are nucleoside analogs and representative of anticancer drugs used in a large number of clinical trials. After phosphorylation, they are incorporated into DNA and covalently link with MTase. As a result, MTase was inactivated (Lyko and Brown, 2005; Goffin and Eisenhauer, 2002). Before studying the inhibition effects of the anticancer drugs on M.SssI, we first evaluated the influence of the inhibitors on BstUI activity. The experiments were performed by cleaving the ds-DNA with the endonuclease for 2 h in the presence of different concentrations of the inhibitors (5-Aza, 5-Aza-dC, or 3-TC). The DPV signals were recorded and compared with that obtained for cleavage in the absence of the inhibitors. The results indicated that the three drugs have negligible influence on the activity of BstUI when the concentrations of 3-TC, 5-Aza and 5-Aza-dC are less than 10, 5, and 2 μM, respectively (results not shown). We then evaluated the inhibitory effects of the three drugs on M.SssI activity by comparing the DPV signal under various concentrations of the drug. The relative activity of M.SssI is estimated using the equation (Ji et al., 2014; Zeng et al., 2013; Deng et al., 2014):

$$\text{Relative activity} = \frac{I_2 - I_0}{I_1 - I_0} \quad (3)$$

where I_0, I_1, and I_2 are DPV signals of the ds-DNA untreated M. SssI (0 U/mL M.SssI), treated with 160 U/mL M.SssI, and treated with 160 U/mL M.SssI in the presence of various dose of inhibitors, respectively. In the methylation step, the DPV signal was recorded after the methylated DNA was cleaved with BstUI for 2 h then hybridized with helper DNA3&DNA4 for 2 h. The activity of the M. SssI decreased with increasing doses of 5-Aza, 5-Aza-dC, and 3-TC (Fig. 4A–C), showing significant dose-dependent inhibition. It is

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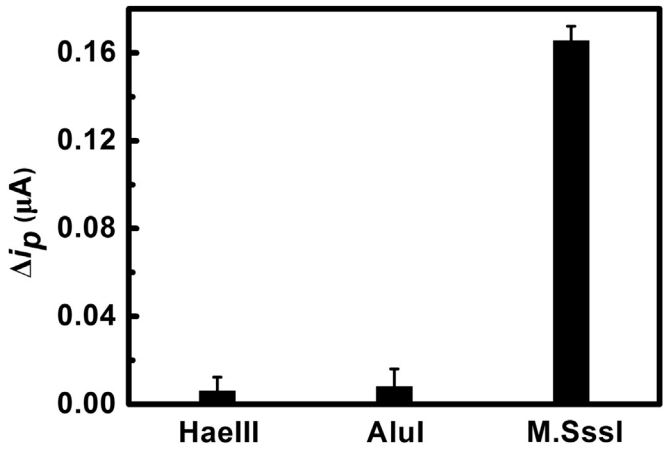

Fig. 4. Dose-dependent inhibition of M.SssI activity by three anticancer drugs (A) 5-Aza, (B) 5-Aza-Dc, and (C) 3-TC. (D) The relative activity of M.SssI in the presence of 1 μM of the each inhibitor. Error bars: SD, n=3.

worth noting that 5-Aza-dC exhibited the highest inhibition efficiency of the three selected drugs (Fig. 4D). This is in accordance with the fact that 5-Aza-dC does not need to be modified to a deoxy form and can be more readily incorporated into DNA (Lyko and Brown, 2005). The IC_{50} value, the inhibitor concentration required to reduce enzyme activity by 50%, was acquired from the plots of relative activity of M.SssI versus the inhibitors concentration and was found to be ~0.3, 2.5, and 2.6 μM for 5-Aza-dC, 5-Aza, and 3-TC, respectively. These results indicate that the developed method has potential application in studying the inhibition effects of anticancer drugs on MTase and for screening MTase inhibitors. It is noteworthy that this study on pharmacological inhibition of DNA MTase can lead to a broad range of therapeutic applications, including use as antibiotics and anticancer therapeutics.

4. Conclusion

In summary, we have described a sensitive method for M.SssI activity assay by coupling a DNA superstructure and site-specific cleavage by BstUI. This assay can determine as low as 0.025 U/mL (at a signal/noise of 3) of M.SssI with a linear range of 0.5–120 U/mL. Compared with traditional electrochemical methods, the present method does not need tedious labeling or multistep procedures. The method can be used for evaluation and screening of inhibitors of MTase and will be useful in the discovery of anticancer drugs. However, since we use 2 mm gold electrode but not micro-electrode, the proposed method cannot conveniently detect DNA methyltransferase activity in cells or in vivo.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.07.103.

References

Baylin, S.B., Herman, J.G., 2000. Trends Genet 16, 168–174.
Belinsky, S.A., Nikula, K.J., Baylin, S.B., Issa, J.P., 1996. Proc. Natl. Acad. Sci. 93, 4045–4050.
Branciamore, S., Chen, Z.X., Riggs, A.D., Rodin, S.N., 2010. Proc. Natl. Acad. Sci. 107, 15485–15490.
Carrascosa, L.G., Sina, A.A.I., Palanisamy, R., Sepulveda, B., Otte, M.A., Rauf, S., Shiddiky, M. J.A., Trau, M., 2014. Chem. Commun. 50, 3585–3588.
Chen, X., Hong, C.Y., Lin, Y.H., Chen, J.H., Chen, G.N., Yang, H.H., 2012. Anal. Chem. 84, 8277–8283.
Chen, Y., Wang, Q., Xu, J., Xiang, Y., Yuan, R., Chai, Y.Q., 2013. Chem. Commun. 49, 2052–2054.
Dammann, R., Li, C., Yoon, J.H., Chin, P.L., Bates, S., Pfeifer, G.P., 2000. Nat. Genet. 25, 315–319.
Das, P.M., Singal, R., 2004. J. Clin. Oncol. 22, 4632–4642.
Deng, H.M., Yang, X.J., Yeo, S.P.X., Gao, Z.Q., 2014. Anal. Chem. 86, 2117–2123.
Fraga, M.F., Uriol, E., Diego, L.B., Berdasco, M., Esteller, M., Canal, M.J., Rodriguez, R., 2002. Electrophoresis 23, 1677–1681.
Furst, A.L., Muren, N.B., Hill, M.G., Barton, J.K., 2014. Proc. Natl. Acad. Sci. USA 111, 14985–14989.
Gao, C.Y., Li, H.N., Liu, Y.J., Wei, W., Zhang, Y.J., Liu, S.Q., 2014. Analyst 139, 6387–6392.
Ge, Z.L., Lin, M.H., Wang, P., Pei, H., Yan, J., Shi, J.Y., Huang, Q., He, D.N., Fan, C.H., Zuo, X.L., 2014. Anal. Chem. 86, 2124–2130.
Goffin, J., Eisenhauer, E., 2002. Ann. Oncol. 13, 1699–1716.
Issa, J.P.J., Vertino, P.M., Wu, J., Sazawal, S., Celano, P., Nelkin, B.D., Hamilton, S.R., Baylin, S. B., 1993. J. Natl. Cancer Inst. 85, 1235–1240.
Ji, L.J., Cai, Z.W., Qian, Y.D., Wu, P., Zhang, H., Cai, C.X., 2014. Chem. Commun. 50, 10691–10694.
Kang, J.S., Meier, J.L., Dervan, P.B., 2014. J. Am. Chem. Soc. 136, 3687–3694.
Kobayashi, Y., Absher, D.M., Gulzar, Z.G., Young, S.R., McKenney, J.K., Peehl, D.M., Brooks, J.D., Myers, R.M., Sherlock, G., 2011. Genome Res 21, 1017–1027.
Li, W., Wu, P., Zhang, H., Cai, C.X., 2012a. Anal. Chem. 84, 7583–7590.
Li, W., Liu, X.J., Hou, T., Li, H.Y., Li, F., 2015. Biosens. Bioelectron. 70, 304–309.
Li, W., Liu, Z., Lin, H., Nie, Z., Chen, J., Xu, X., Yao, S., 2010. Anal. Chem. 82, 1935–1941.
Li, Y., Huang, C.C., Zheng, J.B., Qi, H.L., 2012b. Biosens. Bioelectron. 38, 407–410.
Liu, H.Y., Xu, S.M., He, Z.M., Deng, A.P., Zhu, J.J., 2013. Anal. Chem. 85, 3385–3392.
Liu, S.N., Wu, P., Li, W., Zhang, H., Cai, C.X., 2011. Chem. Commun. 47, 2844–2846.
Lyko, F., Brown, R., 2005. J. Natl. Cancer Inst. 97, 1498–1506.
Muren, N.B., Barton, J.K., 2013. J. Am. Chem. Soc. 135, 16632–16640.
Mutze, K., Langer, R., Schumacher, F., Becker, K., Ott, K., Novotny, A., Hapfelmeier, A., Höfler, H., Keller, G., 2011. Eur. J. Cancer 47, 1817–1825.
Salyan, M.E.K., Pedicord, D.L., Bergeron, L., Mintier, G.A., Hunihan, L., Kuit, K., Balanda, L. A., Shames, D.S., Minna, J.D., Gazdar, A.F., 2007. Curr. Mol. Med. 7, 85–102.
Shen, Q.M., Han, L., Fan, G.C., Abdel-Halim, E.S., Jiang, L.P., Zhu, J.J., 2015. Biosens. Bioelectron. 64, 449–455.
Skiriute, D., Vaitkiene, P., Asmoniene, V., Steponaitis, G., Deltuva, V.P., Tamasauskas, A., 2013. J. Neurooncol. 113, 441–449.
Song, G., Chen, C., Ren, J., Qu, X., 2009. ACS Nano 3, 1183–1189.
Stains, C.I., Furman, J.L., Segal, D.J., Ghosh, I., 2006. J. Am. Chem. Soc. 128, 9761–9765.
Steel, A.B., Herne, T.M., Tarlov, M.J., 1998. Anal. Chem. 70, 4670–4677.
Steichen, M., Decrem, Y., Godfroid, E., Buess-Herman, C., 2007. Biosens. Bioelectron. 22, 2237–2243.
Su, J., He, X.X., Wang, Y.H., Wang, K., Chen, Z.F., Yan, G.P., 2012. Biosens. Bioelectron. 36, 123–128.
Szyf, M., 2003. Drug Resist. Updat. 6, 341–353.
Torres, A.L., Barrientos, E.Y., Wrobel, K., Wrobel, K., 2011. Anal. Chem. 83, 7999–8005.
Wang, G.F., He, X.P., Wang, B.J., Zhang, X.J., Wang, L., 2012a. Analyst 137, 2036–2038.
Wang, G.F., Huang, H., Wang, B.J., Zhang, X.J., Wang, L., 2012b. Chem. Commun. 48, 720–722.
Wang, M., Xu, Z.N., Chen, L.J., Yin, H.S., Ai, S.Y., 2012a. Anal. Chem. 84, 9072–9078.
Wang, X.Z., Liu, X.L., Hou, T., Li, W., Li, F., 2015. Sens. Actuator, B 208, 575–580.
Wang, Y.H., He, X.X., Wang, K.M., Su, J., Chen, Z.F., Yan, G.P., Du, Y.D., 2013. Biosens. Bioelectron. 41, 238–243.
Wei, X.F., Ma, X.M., Sun, J.J., Lin, Z.Y., Guo, L.H., Qiu, B., Chen, G.N., 2014. Anal. Chem. 86, 3563–3567.
Wu, C.T., Morris, J.R., 2001. Science 293, 1103–1105.
Wu, H.W., Liu, S.C., Jiang, J.H., Shen, G.L., Yu, R.Q., 2012. Chem. Commun. 48, 6280–6282.
Wu, Z., Wu, Z.K., Tang, H., Tang, L.J., Jiang, J.H., 2013. Anal. Chem. 85, 4376–4383.
Xia, F., White, R.J., Zuo, X.L., Patterson, A., Xiao, Y., Kang, D., Gong, X., Plaxco, K.W., Heeger, A.J., 2010. J. Am. Chem. Soc. 132, 14346–14348.
Xing, X.W., Tang, F., Wu, J., Chu, J.M., Feng, Y.Q., Zhou, X., Yuan, B.F., 2014. Anal. Chem. 86, 11269–11274.
Yang, L., Zhang, C.H., Jiang, H., Li, G.J., Wang, J.H., Wang, E.K., 2014. Anal. Chem. 86, 4657–4662.
Yang, W.T., Zheng, P.S., 2014. PLoS One 9, e88827.
Yu, J., Ni, M., Xu, J., Zhang, H.Y., Gao, B.M., Gu, J.R., Chen, J.G., Zhang, L.S., Wu, M.C., Zhen, S. S., Zhu, J.D., 2002. BMC Cancer 2, 29–42.
Yuan, T., Liu, Z.Y., Hu, L.Z., Zhang, L., Xu, G.B., 2011. Chem. Commun. 47, 11951–11953.
Zeng, Y.P., Hu, J., Zhang, C.Y., 2013. Anal. Chem. 85, 6143–6150.
Zhang, L.Q., Wei, M., Gao, C.Y., Wei, W., Zhang, Y.J., Liu, S.Q., 2015. Biosens. Bioelectron. 73, 188–194.
Zhao, H.F., Liang, R.P., Wang, J.W., Qiu, J.D., 2015. Biosens. Bioelectron. 63, 458–464.
Zhou, J.W., Zhang, X.H., Xiong, E.H., Yu, P., Chen, J.H., 2015. Chem. Commun. 51, 5081–5084.
Zhou, Y.L., Xu, Z.N., Wang, M., Sun, B., Yin, H.S., Ai, S.Y., 2014. Biosens. Bioelectron. 53, 263–267.