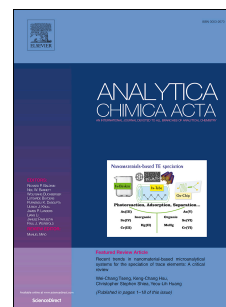


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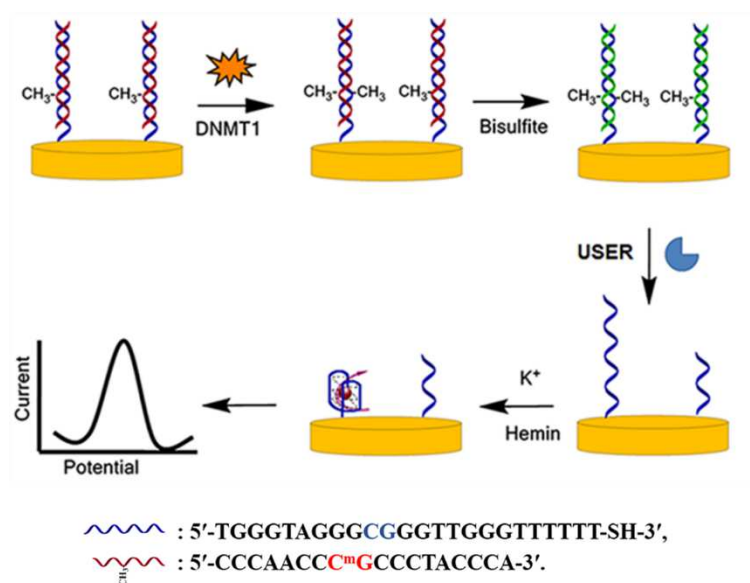
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**Assay of DNA methyltransferase 1 activity based on uracil-specific excision
reagent digestion induced G-quadruplex formation**

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ABSTRACT: DNA methylation catalyzed by DNA methyltransferase plays an important role in many biological processes including gene transcription, genomic imprinting and cellular differentiation. Herein, a novel and effective electrochemical method for the assay of DNA methyltransferase 1(DNMT1) activity has been successfully developed by using uracil-specific excision reagent (USER) induced G-quadruplex formation. Briefly, double stranded DNA containing the recognition sequence of DNMT1 is immobilized on the electrode. Among them, one strand (DNA S1) contains G-rich sequence and a cytosine base, while the supplement strand (DNA S2) contains C-rich sequence and a methylated cytosine. Through the activity of

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DNMT1, the hemimethylated CG recognition sequence of the double stranded DNA are methylated and DNA S2 strand is cleaved and removed after the subsequently treatment with EpiTect fast bisulfite conversion kits and USER, leaving the DNA S1 to form the G-quadruplex-hemin DNAzyme for signal amplification. Under optimal-conditions, the method shows wide linear range of $0.1\text{--}40\text{ U}\cdot\text{mL}^{-1}$ with a detection limit of $0.06\text{ U}\cdot\text{mL}^{-1}$. Furthermore, the inhibition assay study demonstrates that SGI-1027 can inhibit the DNMT 1 activity with the IC₅₀ values of $6\text{ }\mu\text{M}$ in the presence of $160\text{ }\mu\text{M}$ S-adenosylmethionine. Since this method can detect human DNMT1 activity effectively and has successfully been applied in complex biological samples, it may have great potential in the applications in DNA methylation related clinical practices and biochemical researches.

Keywords: Electrochemical biosensor; DNMT1; USER; G-quadruplex.

1. Introduction

DNA methylation plays an important role in many normal cellular processes including gene regulation, X chromosome inactivation and development in mammals [1-3]. In human beings, DNA methyltransferase 1 (DNMT1) is characterized as a maintenance methyltransferase and prefers to hemimethylate DNA at 5'-CG-3' sites [4, 5]. It is proved to be upregulated in tumors and associated with the progression of patients with pancreatic cancer [6, 7]. So it is necessary to develop an effective method for the detection of DNMT1 for early cancer diagnosis and understanding of the carcinogenesis mechanism.

Traditional methods for the assay of DNA methyltransferase activity frequently employed the radioactive-labeling strategy and immune-based detection [8-10]. However, most of them either are involved the use of radioactive materials or labor-intensive. To overcome the shortcomings of the traditional methods, much effort has been made to detect DNA methyltransferase activity using colorimetric[11-14], fluorescent [15-19], and electrochemical methods [20-25]. Among them, electrochemical methods exhibit unique merits of high sensitivity and simplicity of operation, which have attracted much attention in the field of DNA methylation detection. However, most of available electrochemical methods are applicable in the detection of methyltransferase activity in prokaryotic cells. The assay of human DNMT1 involves the exposure of electrode surfaces to greater amounts of protein material due to the larger size and lower activity of these proteins, as compared to bacterial methyltransferases. These proteins can bind to DNA substrate with specificity or non-specificity. And the binding can not be reversed by washing with buffer, which will hinder the cleavage of DNA substrate catalyzed by methylation sensitive restriction endonuclease, resulting in a weak signal [25]. Therefore, it is highly required to develop a novel method for sensitive detection of human DNMT1.

In this work, we have developed a novel electrochemical method to detect DNMT1 activity based on the USER induced G-quadruplex formation [26-30]. Firstly, double stranded DNA (dsDNA) containing recognition sequence of DNMT1 is immobilized on the electrode. Among them, DNA S1 contains G-rich sequence and a cytosine base, while the DNA S2 contains C-rich sequence and a methylated cytosine.

After being treated with DNMT1, the hemimethylated CG recognition sequence is methylated by DNMT1. Upon the treatment of EpiTect fast bisulfite conversion kits [15], cytosine bases of the dsDNA are converted to uracil bases, the methylated cytosine base on the DNA S1 cannot convert. More importantly, most of proteins binding to the DNA substrate are removed after treatment with EpiTect fast bisulfite conversion kits, which makes it easy for the treatment of DNA subsequently[31, 32]. When USER, which contains uracil DNA glycosylase and DNA glycosylase-lyase endonuclease VIII, is dropped on the electrode, uracil DNA glycosylase (UDG) catalyzes the excision of dU forming an abasic site, meanwhile endonuclease VIII breaks the phosphodiester bond of the abasic site and releases the DNA S2. However, the methylated cytosine base on the DNA S1 strand cannot convert to uracil bases and is averted from cleavage. Then, upon addition of hemin, the G-rich sequences form G-quadruplex-hemin DNAzyme, which would catalyze peroxidation reaction to generate amplified signal readout. In the absence of DNMT1, the unmethylated cytosine base on the DNA S1 is converted to uracil bases and cleaved, preventing the formation of G-quadruplex. Therefore, a novel biosensor has been developed to detect DNMT1 activity and screen the inhibitors in eukaryotic cells. This method can be used to determine DNMT1 activity with the detection limit of $0.06 \text{ U} \cdot \text{mL}^{-1}$ and has been successfully applied in complex biological samples, which may be a potential and powerful tool for clinical diagnostics and drug development for cancer in the future.

2. Experimental

2.1. Reagents and materials

All the HPLC-purified oligonucleotide sequences were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and stored in the TE buffer at -20°C in the refrigerator. The base sequences are as follows:

DNA S1: 5'-TGGGTAGGGCGGGTTGGGTTTTTT-SH-3',

DNA S2: 5'-CCCAACCC^mGCCCTACCCA-3'.

Ammonium acetate, and mercaptohexanol (MCH), ethylene diamine tetraacetic acid (EDTA) and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich. S-adenosylmethionine (SAM), USER consists of a mixture of uracil DNA glycosylase (UDG), DNA glycosylase-lyase endonuclease VIII and 10× UDG reaction buffer (200 mM Tris-HCl, 10 mM EDTA, 100 mM NaCl, pH 8.2) were obtained from New England Biolabs Inc. EpiTect fast bisulfite conversion kits were purchased from QIAGEN Co. Ltd (Shanghai, China). DMEM medium was purchased from Sangon Biotechnology Co. Ltd (Shanghai, China). Fetal calf serum was obtained from Sangon Biotechnology Co. Ltd (Shanghai, China). Nuclear protein extraction kit was purchased from Sangon Biotechnology Co. Ltd (Shanghai, China). Hemin, hydroquinone(HQ), H₂O₂ and EDTA were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). The buffer solutions used in this work included TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0), DNA hybridization buffer (10 mM Tris-HCl, 1.0 mM EDTA, and 1.0 M NaCl, pH 7.4), DNA immobilization buffer (10 mM Tris-HCl, 1 mM EDTA, 10 mM TCEP and 0.1 M NaCl, pH 7.4) and electrochemistry determination buffer (0.1 M PBS containing 1.0 mM H₂O₂ and 0.2 mM HQ, pH 7.4).

0.1 M PBS (pH 7.4) was prepared by dissolving 0.2 g NaH_2PO_4 , 2.2 g Na_2HPO_4 and 8.5 g NaCl in 1000 ml doubly distilled water. For all experiments, aqueous solutions purified by Milli-Qplus 185 ultrapure waters system (Barnstead, Bedford, MA) was used. All other chemicals not mentioned here were of analytical reagent grade.

2.2. Instrumentation

All electrochemical measurements were performed on a CHI660D electrochemical workstation (Shanghai Chenhua Instruments Co., Ltd., China) with a conventional three electrode system composed of platinum wire as auxiliary, Ag/AgCl electrode as reference, and a 3 mm-diameter gold electrode as working electrode.

2.3. Cell culture and DNMT1 extraction

MCF-7 cells and Hela cells were cultured in DMEM medium containing 10% fetal calf serum and maintained in a humidified atmosphere with 5% CO_2 at 37 °C. All cells were collected in the exponential phase of growth and harvested from adherent cell culture by trypsinization, followed by washing with cold PBS and then a nuclear protein extraction kit. The cells were incubated for 20 min on ice and then centrifuged at 12 000 rpm (14463 g) for 10 min at 4 °C. Finally, the supernatant was exchanged by a size exclusion spin column into DNMT1 activity buffer. Cell lysate was flash frozen and stored at -80 °C before use.

2.4. DNA immobilization and hybridization

The gold electrode pretreatment involved the following steps[33]. First, the gold electrode ($\phi=3$ mm) was polished with alumina powder (Al_2O_3) of various particle sizes (1.0, 0.3 and 0.05 μm) on silk till a mirror-like smoothness. Then, the electrode

was soaked in piranha solution [$V(H_2SO_4) : V(30\% H_2O_2) = 3 : 1$] for 10 min to eliminate the adsorbed organic matter, and rinsed thoroughly with deionized water, followed by immersed into 50% nitric acid for 30 min. Then, the electrode was sonicated in ethanol and deionized water for 5 min separately. After being dried with nitrogen blowing, the electrode was swept in 0.5 M H_2SO_4 by cyclic voltammetry (CV) from 0 to 1.6 V until stable signal was obtained.

For probe immobilization, 10 μL of probe immobilization buffer containing 2.0 μM thiol-capped DNA S1 was dripped on gold electrode surface and maintained for 12 h at humid conditions[34]. Then, the modified electrode was rinsed three times with DNA immobilization buffer. After that, the electrode was treated with 10 μL of Tris-HCl (10 mM) containing 1 mM MCH and kept for 15 min to hold a good orientation of probe DNA for its good recognition ability. The hybridization experiments were carried out by dripping 10 μL of DNA hybridization buffer containing 1.0 μM of DNA S2 on the electrode surface at 37 °C for 2 h. After that, the hybridized electrode was rinsed three times with hybridization buffer to remove the unhybridized target DNA and dried with nitrogen blowing.

2.5. DNA methylation and bisulfite treatment

The hemimethylation DNA S1/DNA S2 was performed methylation at 37 °C for 2 h in 50 mM Tris-HCl buffer (pH 7.8) containing 160 μM SAM, 1 mM EDTA, 1 mM dithiothreitol (DTT), 5 % glycerol and various concentrations of DNMT1 (from 0 to 40 $unit \cdot mL^{-1}$)[35]. Then the electrode was rinsed three times with 10 mM PBS (pH 7.4) and immersed in 50 μL bisulfite reaction buffer containing 34 μL bisulfite

solution, 10 μL RNase-free water, and 6 μL DNA protect buffer at 60 $^{\circ}\text{C}$ for 30 min. After that, the electrode was rinsed three times with 10 mM PBS and used immediately or stored at -4 $^{\circ}\text{C}$.

2.6. Digestion by USER and formation of G-quadruplex-based DNzyme

50 μL 1 $\times$ USER reaction buffer (50 mM Potassium acetate, 20 mM Tris-acetate, 10 mM Magnesium acetate, 100 $\mu\text{g}\cdot\text{mL}^{-1}$ BSA, pH 7.9) and a known concentration of USER (2.0 $\text{U}\cdot\text{mL}^{-1}$) or cell lysate were added to the gold electrode containing a duplex DNA substrate. The mixture was incubated at 37 $^{\circ}\text{C}$ for 30 min to allow the base cleavage reaction to take place. After that, the electrode was rinsed with 0.1 M PBS to remove the cleaved DNA. Then, 10 μL hemin solution (25 mM HEPES, 50 mM KCl, 200 mM NaCl, 12.5 mM MgCl_2) was added on to the electrode surface at 37 $^{\circ}\text{C}$ for 3 h. Due to the guanine-rich sequences of DNA S1, G-quadruplex-based DNzyme was formed.

2.7. Inhibition of DNMT1 activity

To study the inhibition effect of SGI-1027 on DNMT1 activity, the hemimethylation DNA S1/DNA S2 methylation was performed at 37 $^{\circ}\text{C}$ for 2 h in 50 mM Tris-HCl buffer (pH7.8) containing 160 μM SAM, 1mM EDTA, 1 mM dithiothreitol (DTT), 5% glycerol and 60 $\text{U}\cdot\text{mL}^{-1}$ DNMT1 and various concentrations of inhibitors. The inhibition efficiency (%) was evaluated as follows: $\text{Inhibition} = \frac{I_3 - I_2}{I_3 - I_1} \times 100\%$ [36]. I_1 was obtained after the S1/S2 hybrids treated with bisulfite and USER. I_3 was obtained after the S1/S2 hybrids treated with DNMT1, bisulfite and USER. I_2 was the inhibited current of H_2O_2 -hydroquinone system in the presence of

inhibitor.

2.8. Electrochemical measurements

Differential pulse voltammetry (DPV) was performed in a certain volume of 0.1 M PBS (pH 7.4) containing 1.0 mM H₂O₂ and 0.2 mM HQ with the scan range from -0.1 to 0.2 V. Electrochemical impedance spectra (EIS) was performed in 5.0 mM [Fe(CN)₆]^{3-/4-} solution containing 0.1 M KCl with the frequency from 10 to 10⁵ Hz. The electrochemical measurements were all carried out at room temperature.

3. Results and discussion

3.1. Principle of the biosensor

Please insert Scheme 1 here.

The mechanism of the sensing system is depicted in scheme 1. In this design, a signal probe (DNA S1: 5'-TGGGTAGGGCGGGTTGGG TTTTTT-SH-3') is self-assembled on the electrode via Au-S bonding primarily, which consists of G-rich sequence and an only cytosine base. After hybridization with its complement ssDNA (DNA S2: 5'-CCCAACCC^mGCCCTACCCA-3') which contains C-rich sequence and a methylated cytosine, the double stranded DNA (dsDNA) containing recognition sequence of DNMT1 is formed on the electrode surface. After addition of DNMT1, the hemimethylated CG recognition sequence is methylated by DNMT1. Upon the treatment of EpiTect fast bisulfite conversion kits, multiple unmethylated cytosine bases of the DNA S2 are converted to uracil bases, which are cleaved and released to the solution when USER is dripped on the gold electrode. However, methylated cytosine bases on the DNA S1 cannot be converted to uracil

bases. Thus, only the DNA S1 remains on the electrode surface. After addition of hemin in the system, hemin can intercalate into the G-quadruplex structure. Subsequently, a hemin/G-quadruplex DNAzyme is formed, which may significantly improve the catalysis of H_2O_2 by oxidation of hydroquinone, resulting in an obvious reduction peak current of benzoquinone for the assay of DNMT1 activity. However, in the absence of DNMT1, the unmethylated cytosine base of the DNA S1 is converted into uracil base when treated with EpiTect fast bisulfite conversion kits, which is then cut and released to the solution after treated with USER. Therefore, DNA S1 cannot bind hemin to form G-quadruplex. This electrochemical method offers a general and quantitative approach for sensitive detection of DNMT1 activity.

3.2. Characterization of the modification process of the electrode

Please insert Figure 1 here.

In order to characterize the successfully preparation of the modified electrodes, EIS is performed on different modified electrodes in 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution containing 0.1 M KCl. As shown in Figure 1, EIS of the bare electrode is nearly a straight line (curve a). A small semicircle is obtained after self-assembly of thiol-terminated capture DNA S1 (curve b), which characterizes the immobilization of the DNA S1 on the electrode surface. When DNA S2 is hybridized with DNA S1, a big semicircle is observed (curve c). Afterwards, the diameter of the semicircle evidently decreases when the DNA S2/DNA S1 is treated with DNMT1, bisulfite, and USER (curve d). It can be ascribed to the digestion effect of USER towards uracil bases of the DNA. There is a small semicircle (curve e) in the absence of DNMT1,

demonstrating that DNA S2/DNA S1 are all digested by the USER.

3.3. Detection feasibility of DNA methylation

Please insert Figure 2 here.

DPV is selected to investigate the electrochemical behaviors of different electrodes. As shown in Figure 2, in the presence of DNMT1, a significant current signal is observed indicating the occurrence of the methylation of DNA S1/DNA S2 (curve e). However, in the absence of DNMT1, there is much lower current signal (curve b). The reason is clear, because in the absence of DNMT1, the methylation of DNA S1/DNA S2 does not occur. Therefore, no G-quadruplex structure is formed, thus no electrochemical signal amplification can be achieved. Furthermore, in the absence of DNA S2 (curve a), sodium bisulfite (curve c) or USER (curve d), small electrochemical signals are observed, which clearly demonstrate that DNA S2, sodium bisulfite and USER are necessary to achieve significantly amplified signals. Therefore, the above results indicate the feasibility of this method for the detection of DNMT1 activity.

3.4. Optimization of assay conditions

The electrochemical performance of the sensor would be influenced by several factors, such as the concentration of the USER, and the incubation time of hemin. To obtain the best analytical performance, the effects of the concentrations of USER are investigated. As can be seen from Figure S1 A, with the increasing concentrations of USER, the current intensity increases and reaches a maximum at two units per μL . Thus, two units per μL of USER are chosen for the following experiments.

In addition, the current increases significantly with the hemin incubation time from 1 to 3 h. Then, the current decreases slightly from 3 to 3.5 h. The result indicates that excessive incubation time might hinder electron transfer from benzoquinone to the electrode. So, 3 h is adopted as the optimal hemin incubation time (Figure S1B).

3.5. Assay of DNMT1 activity

Please insert Figure 3 here.

Under the optimized conditions, the activity of DNMT1 is investigated by DPV in 0.1 M PBS containing 1.0 mM H₂O₂ and 0.2 mM HQ. As can be seen in Figure 3, the reduction peak current increases with the increasing DNMT1 concentration ranged between 0.1-40 U·mL⁻¹. The linear relationship between the logarithm of DNMT1 concentration and the reduction peak current is obtained as $I (\mu\text{A}) = 6.325 \lg X(\text{U} \cdot \text{mL}^{-1}) + 7.589$ ($R^2=0.993$) and the detection limit is 0.06 U·mL⁻¹ (S/N=3). This result demonstrates that the biosensor has a good sensitivity for DNMT1 determination.

3.6. Reproducibility and selectivity of the DNMT1 biosensor

Please insert Figure 4 here.

In order to test the reproducibility of this DNMT1 biosensor, five parallel electrodes incubated with 1 U·mL⁻¹ DNMT1 are prepared for this determination. The prepared electrodes demonstrate similar electrochemical signals and an acceptable relative standard deviation (RSD) of 4.2 %, indicating the good reproducibility of the DNMT1 biosensor.

To investigate the selectivity of the method, Dam MTase is selected as an interference enzyme to estimate the selectivity of this method. Due to the specific site recognition of DNMT1 toward its substrate, the method can easily discriminate

DNMT1 from Dam MTase. As shown in Figure 4, significant peak current is observed in the presence of DNMT1. In contrast, no distinct peak current is observed in the presence of Dam MTase. These results demonstrated that the amplified strategy for DNMT1 activity exhibited a good selectivity.

3.7. Analysis of complex biological samples

Please insert Figure 5 here.

To further demonstrate the potential of the present method for practical applications, the sensor is applied for direct detection of DNMT1 in human serum. Each recovery of serum is determined by comparing the results obtained before and after the addition of standard DNMT1 to the serum samples. The results are listed in Table S1. The recoveries of DNMT1 in five samples are all found to be 98.2%-107.0%, which validates the reliability and practicality of this method.

The DNMT1 activity in cell lysates is subsequently evaluated by the biosensor. As illustrated in Figure 5, the DPV peak currents increase monotonically with the number of MCF-7 cells and Hela cells[35]. While the DPV peak current of 10^4 normal cells (HEK-293) is nearly the same as that for the blank control. This result is in accordance with the fact that, with increasing cell number, more DNAs are methylated by DNMT1. Thus, more hemin/G-quadruplex DNAzyme are formed, resulting in a strong peak current. Which is in consistent with the conclusion that DNMT1 is overexpressed in most human tumors.

3.8. Influence of inhibitors on DNMT1 activity

Please insert Figure 6 here.

The DNMT1 plays an important role in eukaryotes. Thus the pharmacological

inhibitors of DNMT1 have promising applications for antibiotics and anticancer therapeutics. To further demonstrate the application of our method in the screening of the DNMT1 inhibitors, we have used SGI-1027, a well-known DNMT1 inhibitor to study the influence of an inhibitor on DNMT1 detection, which can inhibit DNMT1 by competing with SAM in the methylation reaction. As shown in Figure 6, with the increasement of the inhibitor concentration, the peak currents are gradually decreased. The SGI-1027 is found to inhibit 50% DNMT1 activity with 6 μM , which is consistent with previous reports.

Please insert Table 1 here

4. Conclusions

In conclusion, we have developed a novel and effective method for the detection of DNMT1 activity based on USER digestion induced G-quadruplex formation utilizing electrochemical technique in eukaryotic cells, which avoids the interference of protein binding to DNA substrate with specificity or non-specificity in other electrochemical methods. Taking advantage of the G-quadruplex-hemin DNAzyme, the method could detect DNMT1 activity sensitively with a lower detection limit of $0.06 \text{ U} \cdot \text{mL}^{-1}$ (Table 1)[37]. Moreover, the method has successfully been applied in complex biological samples and been used to screen the inhibitor. Given the attractive analytical characteristics, the method may be a potential and powerful tool for early cancer detection and treatment in the future.

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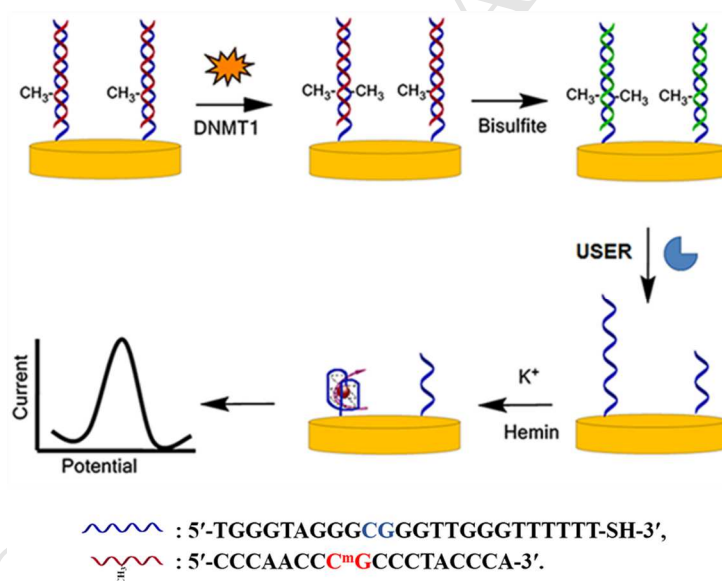
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Scheme 1. Illustration of the DNMT1 activity assay principle

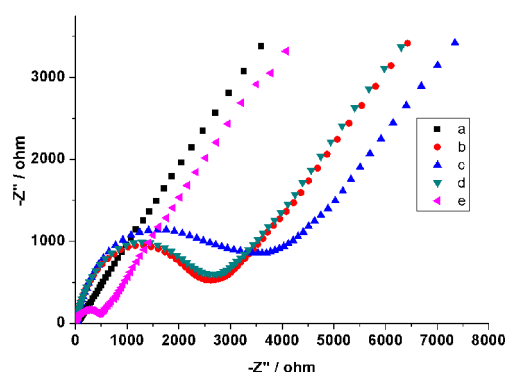


Figure 1. EIS of bare gold electrode (a), the electrode modified with DNA S1 (b), the electrode modified with DNA S2 /DNA S1 (c), the modified electrode treated with DNMT1, sodium bisulfite, USER (d), and the modified electrode treated with sodium bisulfite and USER (e).

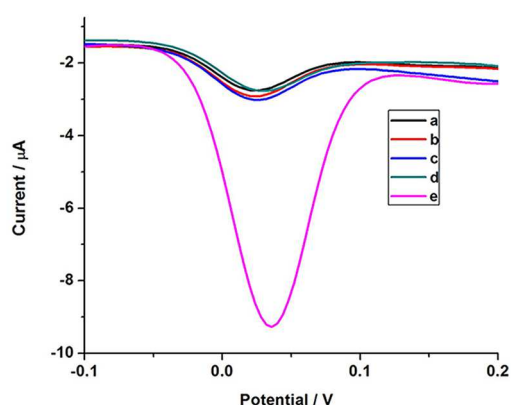


Figure 2. DPV curves for DNA S1 modified gold electrode treated successively with DNMT1, sodium bisulfite and USER (a), DNA S1/DNA S2 hybrids modified electrode treated successively with sodium bisulfite and USER (b), the S1/S2 hybrids treated successively with DNMT1 and USER (c), the S1/S2 hybrids treated successively with DNMT1 and sodium bisulfite (d), the S1/S2 hybrids treated successively with DNMT1, sodium bisulfite and USER (e) in 0.1 M PBS (pH 7.4)

containing 1.0 mM H₂O₂ and 0.2 mM HQ.

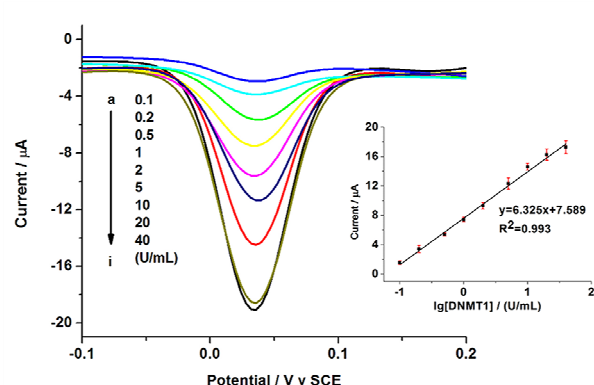


Figure 3. DPV curves for different concentrations of DNMT1 ($\text{U} \cdot \text{mL}^{-1}$): 0.1(a), 0.2 (b), 0.5(c), 1(d), 2(e), 5(f), 10(g), 20(h), and 40 $\text{U} \cdot \text{mL}^{-1}$ (i) in 0.1 M PBS containing 1.0 mM H₂O₂ and 0.2 mM HQ. The inset is the calibration curve. Error bars indicate standard deviation of three independent experiments.

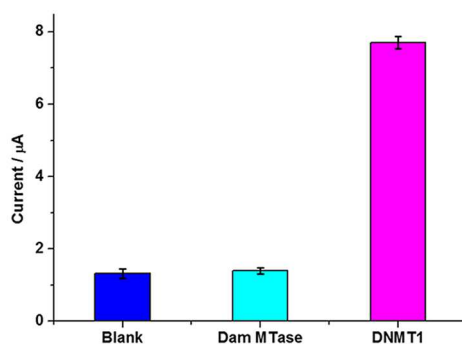


Fig.4. Comparison of the DPV peak currents in the presence of 1.0 $\text{U} \cdot \text{mL}^{-1}$ DNMT1 and 10 $\text{U} \cdot \text{mL}^{-1}$ Dam MTase respectively, in which blank indicates the condition in the absence of Dam MTase as well as DNMT1. The error bars represent the standard deviation of three measurements.

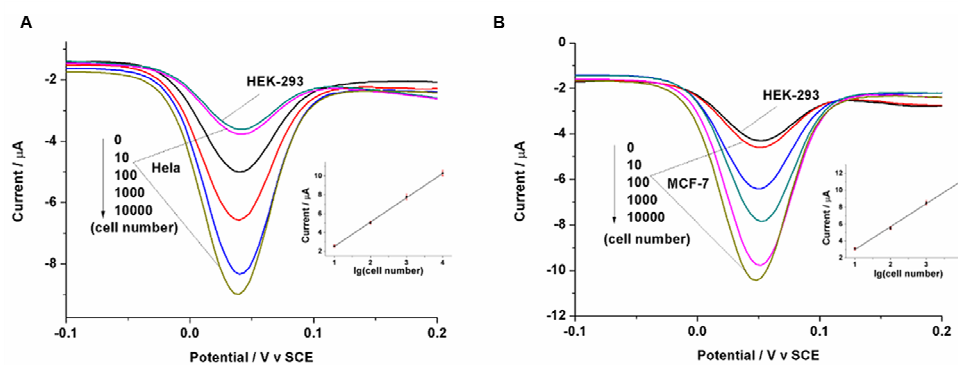


Fig.5. (A), (B) DPVs obtained in detecting Hela cells and MCF-7 cells (0, 10, 100, 1000, 10000 cells). The inset is relationship between the signal response and Hela cells, MCF-7 respectively, with error bars representing standard deviation (n=3).

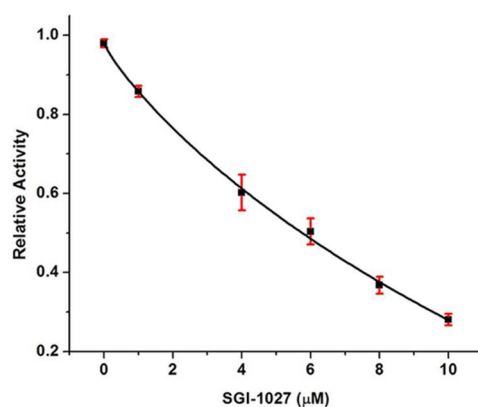
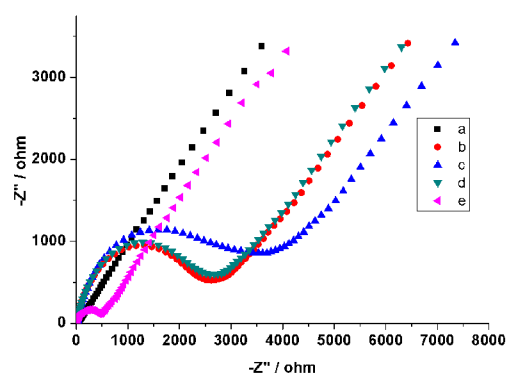
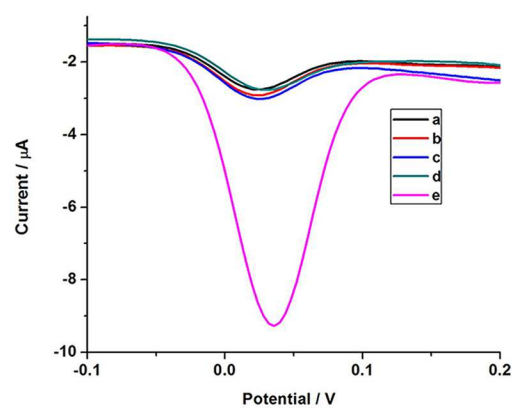


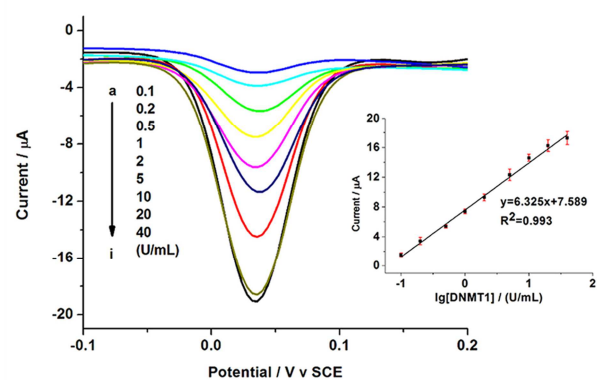
Fig.6. The inhibition effect of SGI-1027 on DNMT1 activity.

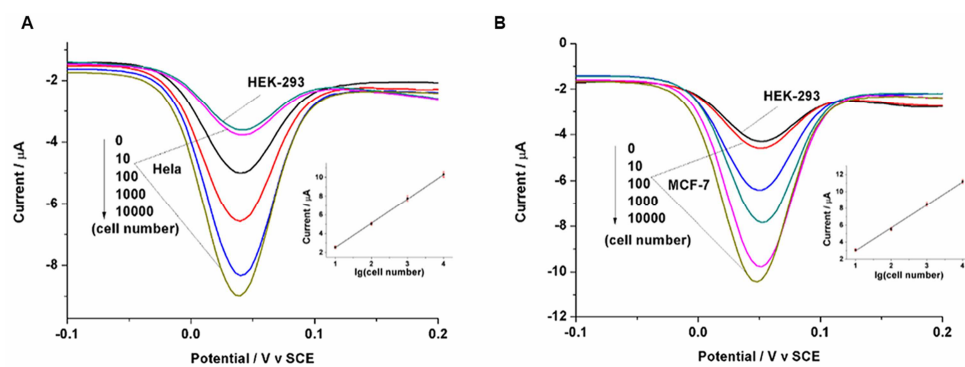
Table 1. A comparison of the method with previously reported ones.

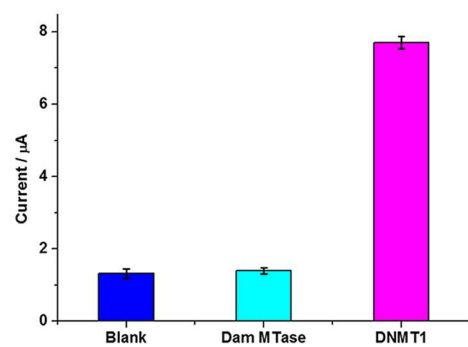
Strategy	Detection method	Detection limit (U·mL ⁻¹)	Linear range (U·mL ⁻¹)	Reference
Coupling AuNPs -DNA Complexes with Ru(III)	DPV	0.3	1-40	[37]
HRP mimicking DNAzyme	DPV	0.06	0.1-40	This work

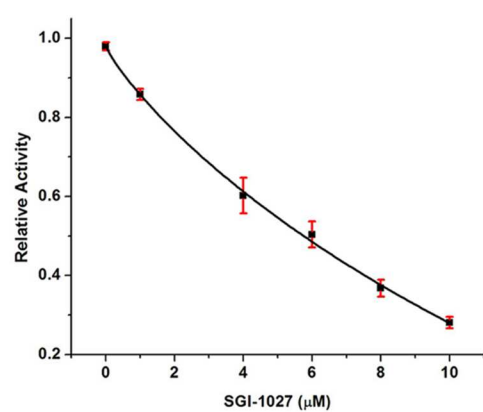


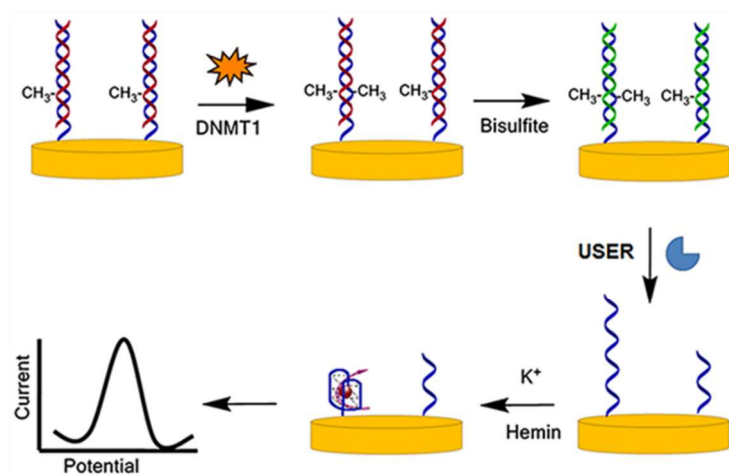












: 5'-TGGGTAGGG**CG**GGTTGGGTTTTT-SH-3',
 : 5'-CCCAACCC**C^m**GCCTACCCA-3'.

Highlights:

- A novel electrochemical method for the assay of DNA methyltransferase 1(DNMT1) activity and its inhibitor screening is developed.
- G-quadruplex is used for signal amplification skillfully.
- DNMT1 activity is detected effectively in complex biological samples.
- This method may have potential applications in clinical practice.