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DNA methyltransferase detection based on digestion triggering the combination of poly adenine DNA with gold nanoparticles

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ABSTRACT

DNA methyltransferase (MTase) has received a large amount of attention due to its catalyzation of DNA methylation in both eukaryotes and prokaryotes, which has a close relationship to cancer and bacterial diseases. Herein, a novel electrochemical strategy based on Dpn I digestion triggering the combination of poly adenine (polyA) DNA with a gold nanoparticles functioned glassy carbon electrode (AuNPs/GCE), is developed for the simple and efficient detection of DNA MTase and inhibitor screening. Only one methylene blue (MB)-labeled DNA hairpin probe and two enzymes are involved in this designed method. In the presence of Dam MTase, the hairpin probe can be methylated and then cleaved by the restriction endonuclease. Thus, a MB-labeled polyA signal-stranded DNA product is introduced to the surface of AuNPs/GCE through the effect between polyA and AuNPs, resulting in an obvious electrochemical signal. On the contrary, in the absence of Dam MTase, the DNA probe cannot be cleaved and a relatively small electrochemical response can be observed. As a result, the as-proposed biosensor offered an efficient way for Dam MTase activity monitoring with a low detection of 0.27 U/mL, a wide linear range and good stability. Additionally, this assay holds great potential for further application in real biological matrices and inhibitors screening, which is expected to be useful in disease diagnosis and drug discovery.

1. Introduction

The methylation of DNA is the most well-known epigenetic modification (Jeltsch et al., 2007) and famous as a critical process existing in both prokaryotes and eukaryotes (Rosi and Mirkin, 2005). It is reported that aberrant DNA methylation is associated with a series of diseases (Jaenisch and Bird, 2003; Jeltsch et al., 2007), which is carried out by DNA methyltransferase (MTase) that catalyzes a transfer of a methyl group from S-adenosyl-L-methionine (SAM) to adenine or cytosine in the recognition sequences (Lee et al., 2010). Due to the importance of DNA MTase in the methylation process, it is regarded as a new cancer biomarker (Erova et al., 2006; Tsankova et al., 2007). Thus, the detection of DNA MTase together with screening of inhibitors is essential for clinical diagnostics and drug development.

So far, some traditional assays for detection of DNA MTase, mainly including radioactive labeling (Adams et al., 1991), high performance liquid chromatography (HPLC) (Johnston et al., 2005; Lopez Torres et al., 2011), gel electrophoresis (Bergerat et al., 1991) and polymerase chain reaction (PCR) (Brandes et al., 2005; Yang et al., 2004), have been established. Unfortunately, these methods have many disadvantages, like time-consuming, laborious, requiring isotope labeling and insensitive. In order to overcome the intrinsic drawbacks, several fascinating strategies have been developed in recent years, such as fluorescence assay (Liu et al., 2015b), colorimetric approaches (Zhao et al., 2014), chemiluminescence methods (Zhao et al. 2015), photoelectrochemical ways (Yang et al., 2015) and electrochemical biosensors (Wang et al., 2013). Among them, electrochemical biosensors have received a large amount of attention because of its attracting abilities of simple, inexpensive and high sensitive. For example, Yin et al. (2013) designed an assay of DNA MTase based on methyl binding domain (MBD) proteins and coomassie brilliant blue G250 (CBB-G250). Last year, a sensitive and selective electrochemical method based on using DNA-modified gold nanoparticles (AuNPs) as signal amplification unit and methylene blue (MB) as electrochemical indicator has been developed for detection of DNA MTase by Jing's group (Jing et al., 2014). However, these methods required a fussy immobilization to the electrode between Au and thiol modified DNA as well as several kinds of DNA sequences for constructing the biosensor. Therefore, to develop simple, efficient and economical methods for DNA MTase is still a challenge.

Recently, a study of the poly adenine (polyA) single-strand sequences preferential adsorb AuNPs with high affinity has been reported (Pei et al., 2012). Inspired by this unique work, herein, we developed a simple method for assay of DNA MTase based on Dpn

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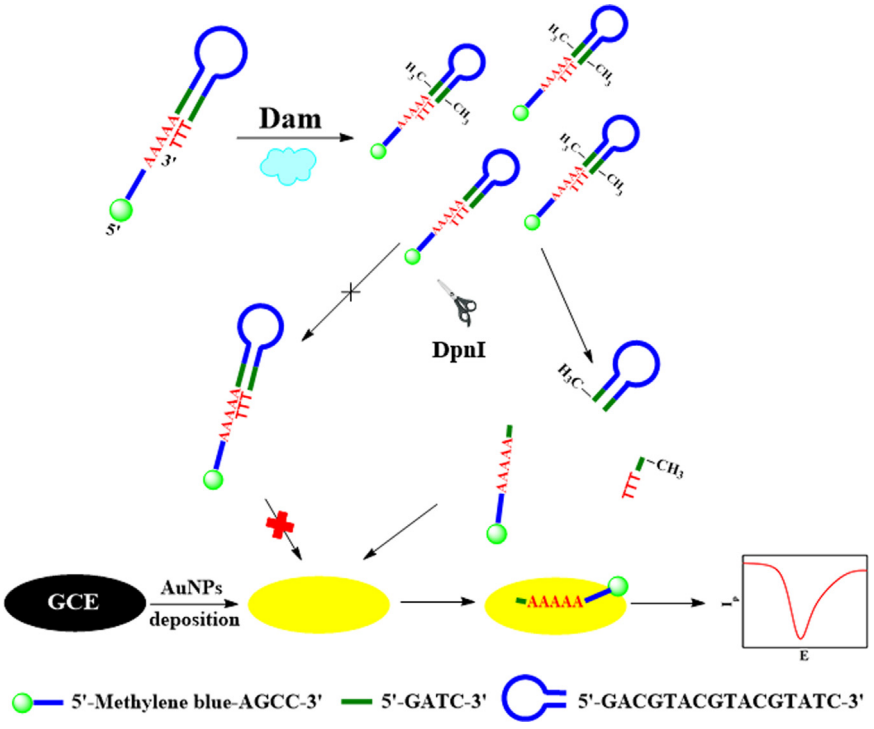

Scheme 1. The principle of the developed method for DNA MTase analysis.

I digestion triggering of the combination of polyA DNA with a gold nanoparticles functionalized glassy carbon electrode (AuNPs/GCE). Only one MB-labeled DNA hairpin probe and two enzymes are involved in this efficient strategy. In addition, due to the high affinity between polyA and AuNPs, the detection can be carried out without fussy immobilization before methylation. What's more, this assay is potential for further application in real biological matrices and inhibitors screening.

The mechanism of the system is illustrated in Scheme 1. In this experiment, only one DNA strand is involved. The hairpin DNA probe is particularly designed to contain an electroactive substance (MB) and two functional domains. One is the polyA sequences which can be served as an effective replacement of thiol for preferential binding with the AuNPs when it is a single strand. The other (the green part) is the recognition site (5′-GATC-3′) for Dam MTase (Liu et al., 2010), a DNA methyltransferase which can facilitate the exchange of a methyl group from SAM to the target adenine. Besides, the methylation-sensitive restriction endonuclease Dpn I is also involved in the system. In the presence of Dam MTase and Dpn I, the hairpin probe will be methylated and then cleaved into two parts (a neo-hairpin structural DNA and a new hybrid). However, the new hybrid is unstable (Wei et al., 2014). Therefore, this hybrid will be separated into two independent signal-stranded DNA fragments. Subsequently, the MB can be introducted to the suface of the electrode through the effect between polyA and AuNPs, resulting in a DPV signal. At higher activity of Dam MTase, more hairpin probe can be methylated and cleaved, and more MB can be introduced to the electrode, and thus, an obvious response was obtained, offering a quantitative read out of Dam MTase activity.

2. Experimental

2.1. Reagents and materials

MB-modified oligonucleotides was purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China), and the sequences of the hairpin probe was 5′-(MB)-AGC CAA AAA GAT CGA CGT ACG TAC GTA TCG ATC TTT-3′. Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$), tris (hydroxymethyl) aminomethane (Tris), tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Aladdin (Shanghai, China). 5-Fluorouracil was obtained from Sigma-Aldrich (St. Louis, USA). All chemicals were of analytical grade and the solutions were prepared by double distilled water after high pressure steam sterilization.

The Dam MTase, M. *Sss*I MTase, SAM and Dpn I endonuclease were respectively supplied by New England Biolabs (Ipswich, MA) and Fermentas (MD, USA). According to the supplier, Dam MTase was stored at −20 °C in a buffer containing 50 mM KCl, 50 mM Tris–HCl (pH 7.5), 10 mM EDTA, 1 mM dithiothreitol (DTT), 200 μg/mL BSA and 50% glycerol. M. *Sss*I MTase was stored at −20 °C in a buffer containing 10 mM Tris–HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 mg/mL BSA and 50% glycerol. Restriction endonuclease Dpn I was stored in a buffer containing 10 mM Tris–HCl (pH 7.4), 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 500 μg/ml BSA, 50% Glycerol at −20 °C in the refrigerator. The TE buffer containing 10 mM Tris–HCl (pH 8.0) and 1 mM EDTA at −20 °C in the refrigerator.

2.2. Instrumentation

All electrochemical experiments were carried out using a CHI660C electrochemical workstation (Austin, USA). A conventional three-component electrochemical cell was employed in electrochemical measurements, with AuNPs/GCE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the auxiliary electrode. Scanning electron microscope (SEM) was performed on an S-4800 SEM. UV–vis spectra were obtained on a Quawell UV–vis spectrophotometer Q5000 (USA).

2.3. Methylation and cleavage of hairpin probe

The methylation and cleavage of hairpin probe were carried out in 40 μL of reaction mixture containing 1 μM probe DNA, various concentration of Dam MTase, 80 μM SAM, 1 × Dam buffer (50 mM Tris–HCl, 10 mM EDTA, 5 mM 2-mercaptoethanol, pH 7.5), 50 U/mL Dpn I and 1 × NEB buffer (20 mM Tris-acetate, 50 mM Potassium acetate, 10 mM mag-nesium acetate and 100 μg/mL BSA, pH 7.9) at 37 °C for different time (0–480 min). And then the mixture was inactivated at 80 °C for 20 min.

2.4. Preparation of AuNPs modified electrode

The AuNPs modified electrode was prepared according to a reported procedure (Liu et al., 2015a). Briefly, the GCE was carefully polished on a microcloth with 30 nm alumina powder and followed by a period of 3 min sonication in double distilled water, anhydrous ethanol and double distilled water sequentially. Then the electode was modified by AuNPs in a 3 mM $HAuCl_4$ solution containing 0.1 M KNO_3 through the amperometry technique at −0.2 V for 200 s. Finally, the obtained AuNPs/GCE was rinsed with a copious amount of double distilled water and characterized by SEM (Fig. S1 in Supplementary material).

2.5. Electrochemical detection of Dam MTase

For detection, the obtained electrode was incubated with 10 μL of the reaction mixture at humid condition for a varying amount of time (0–12 h). After immobilization, the electrode was washed thoroughly with 0.1 M PBS (pH=7.0) to remove the redundant DNA. Then stable and high signal can be obtained from the differential pulse voltammetry (DPV) measurement in 10 mL PBS (0.1 M, pH=7.0), which was conducted in the presence of MB. Each electrochemical measurement was repeated at least 3 times under the same condition with the following parameters: potential step, 4 mV; pulse amplitude, 50 mV; pulse width, 0.05 s; sample width, 0.0167 s; pulse period, 0.2 s; and quite time, 2 s. What's more, the absorbance of the reaction mixture before and after incubating with the AuNPs/GCE were detected by UV–vis spectroscopy and the adsorption efficiency was shown in Table S3.

2.6. Dam MTase inhibition evaluation

Using 5-fluorouracil, a typical inhibitor assay containing 40 U/mL Dam MTase, 1 μM probe DNA, 80 μM SAM, 1 × Dam buffer, 50 U/mL Dpn I, 1 × NEB buffer and various amounts of inhibitor was performed. The processes were similar to the assay of Dam MTase.

3. Results and discussion

3.1. Feasibility study

To demonstrate the feasibility of the developed method, DPV responses of different reaction mixtures were investigated. As shown in Fig. 1, in the present of Dam and Dpn I, a significant DPV signal was observed (curve a), indicating the occurrence of methylation-induced cleavage of DNA and polyA preferential binding with the AuNPs/GCE. However, no DPV signal was observed on AuNPs modified electrode (curve b). In addition, in the absence of Dam MTase, a weak DPV signal (curve c) was detected due to the inhibition of Dpn I to cleave the hairpin probe. Similarly, when the hairpin probe was only treated with Dam MTase without Dpn I, there is also a weak DPV response (curve d) compared with curve c, indicating the importance of Dpn I on methylation recognition and polyA formation. Therefore, this method can be used for sensitive characterization of Dam MTase activity.

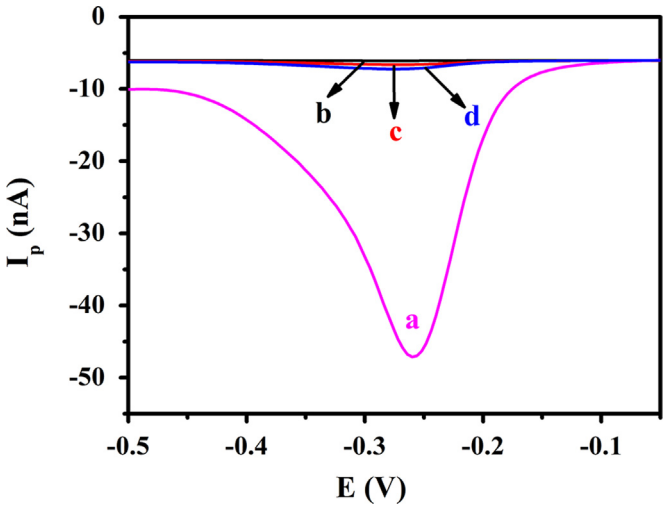

Fig. 1. The DPV responses of the AuNPs/GCE treated with hairpin probe, Dam MTase and Dpn I (a), the AuNPs/GCE (b), the AuNPs/GCE treated with hairpin probe and Dam MTase (c), the AuNPs/GCE treated with hairpin probe and Dpn I (d). All data were recorded in 0.1 M PBS (pH=7.0).

3.2. Selectivity, reproducibility and stability

Selectivity is one of the vital factor to assess the performance of a sensing system. To evaluate the selectivity of the method, M. *Sss*I MTase was selected as an interference enzyme. As seen in Fig. S2, a significant DPV response was observed in the present of Dam MTase. In contrast, a weak DPV response was observed when Dam MTase was replaced by M. *Sss*I MTase. Because the recognition of the interference enzyme was 5′-CG-3′ sequence (Li et al., 2012) which was different from the recognition of Dam MTase. Without methylation, the hairpin probe can not be cleaved and separated into a poly adenine single-strand DNA fragment, resulting that the MB would not be detected. Therefore, this method can easily discriminate Dam MTase from M. *Sss*I MTase.

The other two factor to assess the performance of a sensing system are reproducibility and stability. For assay of reproducibility, we performed the experiment with 40 U/mL Dam MTase for five times with different and freshly prepared electrodes, and the RSD (relative standard deviation) was 5.23%. The stability can be investigated by the change of the DPV response when stored the biosensor for a period of time. Due to the split of DNA, it could retain 96% for 40 U/mL Dam MTase after one week in the refrigerator at 4 °C. These results demonstrated that the strategy for Dam MTase activity detection exhibited good selectivity, satisfactory reproducibility and stability.

3.3. Optimization of assay conditions

To obtain the best analytical performance, the effect of reaction time of Dam MTase and Dpn I, and the immobilization time of polyA on AuNPs/GCE were investigated, respectively. Fig. S3A showed the effect of reaction time of Dam MTase and Dpn I on DPV responses. Increasing the reaction time of methylation and cleaving changed the DPV value more obviously from 0 to 180 min. And then the rise of DPV value slowed down with the increasing of the time when the same cindoncentration of Dam MTase and Dpn I were used. Therefore, reaction time of about 180 min was used in this study.

In addition, the immobilization time of polyA on AuNPs/GCE was also optimized. As shown in Fig. S3B, the DPV response

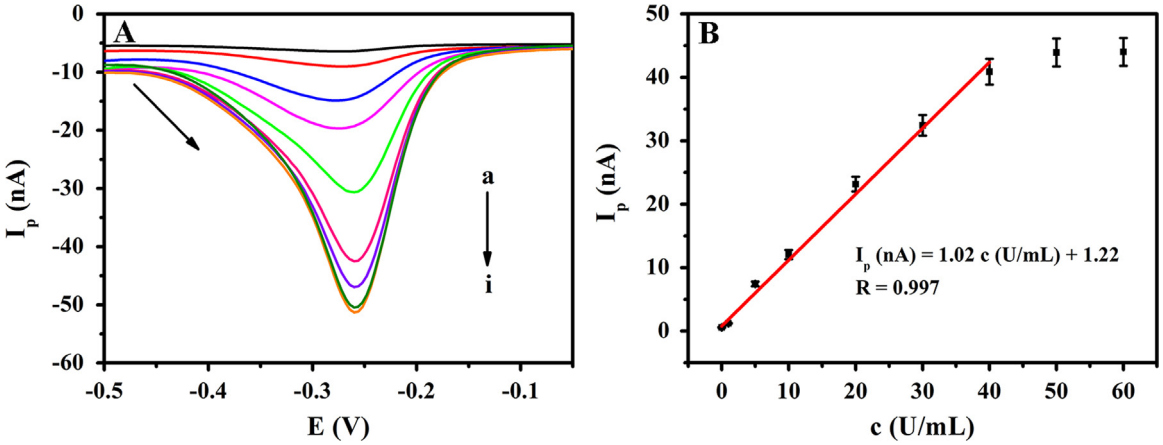

Fig. 2. (A) DPV response in the presence of different concentrations of Dam MTase ranging from 0 to 60 U/mL. (B) Linear relationship between the DPV value and the Dam MTase concentration.

increased gradually with an increase in the immobilization time of polyA and AuNPs/GCE from 0 to 8 h. Further increase of the immobilization time, the DPV response leveld off. This phenomenon might be attributed by the saturation of polyA binding with AuNPs/GCE. Based on the above results, the immobilization time of 8 h was used for the following experiments.

3.4. Activity analysis of Dam MTase

To confirm the ability of the proposed method to detect Dam MTase, a series of different concentrations of Dam MTase were measured (Fig. 2A). As can be seen, as the concentration of Dam MTase increased, large numbers of polyA fragments were produced for binding with AuNPs/GCE, resulting in the increase of the DPV response correspondingly. A linear curve was obtained between the DPV value and concentration of Dam MTase from 0.27 to 40 U/ml (Fig. 2B). The regression equation was I_p (nA)= 1.02c (U/mL)+1.22 with a correlation coefficient of 0.997. The limit of detection of Dam MTase was approximately 0.27 U/mL in terms of the rule of $3\sigma/k$, which was compared with previously reported assays (Table S1 in Supplementary material).

3.5. Analysis of Dam MTase activity in human serum

The applicability in complex biological matrices is a significant challenge for enzyme activity analysis. To further test the practicality of the proposed method, standardized human serum sample (diluted in 1:10 ratio with 50 mM Tris–HCl buffer) spiked with Dam MTase, at three different concentrations of Dam MTase (1, 10 and 40 U/mL), were investigated. As depicted in Fig. S4, it is obvious that the DPV responses for Dam MTase were comparable in both diluted human serum sample and buffer solution. The recoveries and RSD were given in Table S2. These results indicated the potentiality of the way for the Dam MTase analysis in real biological matrices.

3.6. Assay of Dam MTase activity inhibition

To further extend the potential application of this method, the influence of 5-fluorouracil on the activity of Dam MTase was investigated in Fig. S5. It was obvious that the inhibition efficiency increased with the increasing concentration of 5-fluorouracil and may decrease the activity of Dam MTase by about 62.07%. This indicated that Dam MTase activity was inhibited by 5-fluorouracil, which weakened the methylation-cleaved effect and led to weak DPV response. In addition, the IC_{50} value was considered to be about 90 μM, the inhibitor concentration required to reduce enzyme activity by 50%. Therefore, the above results demonstrated that this method can be used to screen inhibitors of Dam MTase with highly sensitive.

4. Conclusion

In summary, we have developed a simple and novel electrochemical strategy for the detection of DNA MTase based on Dpn I digestion and the self-assembly of polyA DNA with AuNPs/GCE. With only one DNA probe and two enzymes involved, this sensitive method is extremely easy in design. As a result, the as-proposed biosensor offered an efficient way for DNA MTase activity monitoring with a low detection limit of 0.27 U/mL, a wide linear range and good stability. In addition, this assay also show a great potential for further application in real biological matrices and inhibitors screening. Movever, the strategy can be extended to other DNA MTase detection and the screening of corresponding inhibitor by changing the corresponding hairpin probe and enzyme, which is expected to be useful in disease diagnosis and drug discovery.

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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.2015.12.100.

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