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Label-free and immobilization-free photoelectrochemical biosensing strategy using methylene blue in homogeneous solution as signal probe for facile DNA methyltransferase activity assay

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ABSTRACT

Photoelectrochemical (PEC) methods have recently witnessed ever expanding application in bioanalysis, but it is still desirable to further simplify the sensing procedures and develop simple and reliable PEC biosensing approaches. Herein, we proposed a truly label-free and immobilization-free PEC sensing platform, utilizing solution-phase methylene blue (MB) as the signal probe, and bare indium tin oxide (ITO) glass as the photoelectrode. Based on the diffusivity difference between free MB molecules and MB intercalated in DNA G-quadruplex, the activity and inhibition of DNA adenine methyltransferase (Dam), a proof-of-concept methyltransferase (MTase), is quantitatively analyzed. By taking advantage of the endonuclease-catalyzed cleavage of the Dam-methylated hairpin DNA probe, as well as the KF polymerase/Nt.*Alw*I endonuclease-aided signal amplification, highly sensitive and specific PEC detection of Dam activity has been achieved. Moreover, this approach can be easily extended to assay other types of MTase by choosing the appropriate methylation-sensitive endonucleases. The as-proposed strategy has also been successfully applied to analyze Dam spiked in human serum samples and to assess the inhibitory effects of antibiotics on Dam activity. More importantly, this label-free and truly immobilization-free PEC biosensing strategy shows additional merits of simplicity and satisfactory repeatability, due to the elimination of both labelling and immobilization procedures, making it a promising candidate for the application in highly sensitive, facile and reliable bioanalysis and drug screening.

1. Introduction

DNA methylation, an epigenetic event found in both prokaryotes and eukaryotes, plays a pivotal role in a variety of cellular processes, including the regulation of gene expression, embryonic development, X-chromosome inactivation, *etc.* (Barras and Marinus, 1989). In the process of DNA methylation, a methyl group is transferred from the donor S-adenosylmethionine (SAM) to the specific adenine or cytosine in the short DNA palindromic sequence under the catalysis of DNA methyltransferases (MTase). It has been found that the abnormality of DNA MTase activity may sabotage normal DNA methylation processes, and aberrant DNA methylation is closely related to neurodevelopmental disorders, genetic disorders, and different types of cancers (Robertson, 2005; Robertson and Wolffe, 2000). So DNA MTase activity are regarded as an important biomarker for these diseases. Moreover, the investigation of DNA MTase activity inhibition by certain inhibitors may provide valuable information for disease treatment and drug discovery. Therefore, it is crucial to realize sensitive and accurate sensing of DNA MTase activity and inhibition.

Conventional methods for DNA MTase activity assays, such as radioactive labeling, high-performance liquid chromatography, polymerase chain reaction, and gel electrophoresis, exhibit the limitations of radioactive hazard, tedious procedures, sophisticated instrumentation and lengthy assay time (Bergerat et al., 1991; Boye et al., 1992; Lennard and Singleton, 1994; van Steensel and Henikoff, 2000). To overcome such drawbacks, a variety of DNA MTase activity assays have been developed, including colorimetric, chemiluminescent, electrochemiluminescent, fluorescent, electrochemical, and photoelectrochemical (PEC) methods (Chen et al., 2018; Li et al., 2015; Muren and Barton, 2013; Rauf et al., 2017; Su et al., 2015; Sun et al., 2018; Wu et al., 2013; Yin et al., 2014; Zhao et al., 2016). Among them, the newly developed PEC sensing strategies have received ever increasing attention. Owning to the total separation of electrochemical readout signal from the excitation light source, PEC sensing exhibits the remarkable

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advantages of low background and potentially high sensitivity comparing to traditional electrochemical detection (Osterloh, 2013; Zhao et al., 2015). Moreover, in contrast to complex and expensive optical detection devices needed for spectroscopic approaches, PEC instrumentation is simple and cheap (Shen et al., 2015; Yang et al., 2015; Zhao et al., 2014). In general, PEC biosensing methods require the immobilization of photoactive materials and/or bio-recognition probes on photoelectrode surfaces to facilitate charge transfer (Osterloh, 2013; Zhao et al., 2018), which, however, complicates the sensing procedure, making them laborious, time consuming and difficult to achieve photoelectrode modification with good repeatability. Further more, the immobilization of bio-recognition probes on photoelectrodes remarkably restricts their configurational freedom because of steric hindrance effects, thus impairing the efficiency and rate of target recognition (Hong et al., 2018; Hou et al., 2018; Lubin et al., 2009). Therefore, it is highly preferable to establish immobilization-free PEC sensing methods.

In the past few years, researches have been carried out to establish PEC biosensing strategies with target recognition and signal amplification carried out in solution phase, thus avoiding immobilization of bio-recognition probes on photoelectrodes (Ge et al., 2016a; Hong et al., 2018; Hou et al., 2016; Lin et al. 2017a, 2017b; Zhang et al., 2018). Among them, the processes of biorecognition and signal amplification were carried out either completely in homogeneous liquid phase (Ge et al., 2016a; Hong et al., 2018; Hou et al., 2016) or *via* the aid of magnetic beads distributed evenly in solution (Lin et al. 2017a, 2017b; Zhang et al., 2018), and highly sensitive and selective detection of proteins, disease biomarkers and toxins have been achieved. However, in the aforementioned approaches, it is still necessary to immobilize pre-synthesized or *in-situ* generated photoactive materials on photoelectrodes, which could be further simplified by banishing the deposition of photoactive probes on photoelectrodes to achieve truly immobilization-free PEC detection.

Very recently, inspired by the homogeneous electrochemical strategies for the assay of a variety of analytes (Ge et al., 2016b; Hou et al., 2015; Liu et al., 2015; Tan et al., 2015; Wei et al., 2014; Xuan et al., 2012), as well as PEC sensing based on photoactive organic dyes (Cooper et al. 1998, 1999; Hao et al., 2016), our group has established a truly immobilization-free diffusivity-mediated PEC sensing strategy (Hou et al., 2018), in which methylene blue (MB) labelled on DNAs was adopted as the photoactive probe, and indium tin oxide (ITO) glass without surface modification as the working electrode. By taking advantage of the diffusivity difference between MB-labelled DNAs and MB-labelled mononucleotides, highly sensitive microRNA assay was achieved *via* T7 exonuclease-assisted signal amplification. Since both target recognition and signal amplification took place in homogeneous solution, and photoelectrode modification is successfully avoided, this truly immobilization-free PEC sensing strategy demonstrates the advantages of simplicity, rapidness and good repeatability. However, in this approach, the DNA probe is labelled with MB, which leaves room to further simplify the procedure and lower the cost. Thus, it is preferable to design both label-free and immobilization-free PEC analysis methods.

Herein, we propose a label-free and immobilization-free PEC sensing strategy, in which MB in homogeneous solution acts as the photoactive probe, and *via* the diffusivity difference between free MB molecules and MB intercalated in DNA G-quadruplex towards ITO photoelectrodes, the activity and inhibition of DNA adenine methyltransferase (Dam), a proof-of-concept MTase, is quantitatively analyzed. In this approach, as shown in Scheme 1, Dam initiates the methylation of a customer-designed DNA hairpin probe and its subsequent cleavage catalyzed by endonuclease *Dpn*I, releasing a single-stranded trigger DNA. With the aid of an assistant DNA, KF polymerase and Nt.*Alw*I endonuclease, large amounts of G-rich single-stranded DNAs are generated, which subsequently fold into G-quadruplexes in the presence of K^+. Upon the intercalation of MB in G-quadruplexes, the diffusivity of MB significantly decreases due to the electrostatic repulsion between the both negatively charged G-quadruplexes and ITO photoelectrode surface, resulting in significantly decreased PEC responses. Therefore, highly sensitive and specific PEC assay of Dam activity and inhibition is accomplished. In this PEC biosensing strategy, in addition to eliminating the immobilization of bio-recognition probes and photoactive materials on photoelectrode, the labelling of DNA probe is also circumvented, which further simplifies the sensing procedures and lowers the cost, making it a valuable approach to be applied in facile, rapid and reliable bioanalysis and drug screening.

2. Experimental section

2.1. Reagents and materials

The details are described in Supporting Information.

2.2. DNA methylation and cleavage by Dpn I

First, 10 μL of 10 μM HP, 5 μL of Dam with different concentrations in 1 × Dam buffer (pH 7.5, 50 mM Tris-HCl, 10 mM NaCl, 10 mM EDTA, 5 mM 2-mercaptoethanol), 2 μL of 3.2 mM SAM, and 5 μL of 800 U/mL *Dpn*I in 1 × NEB buffer 4 (pH 7.9, 50 mM KAc, 20 mM Tris-Ac, 10 mM $MgAc_2$, 1 mM DTT) were mixed. Next, 52 μL of Tris-HCl buffer (pH 7.4, 10 mM Tris, 100 mM NaCl, 10 mM $MgCl_2$, and 200 mM KCl) was added, and the mixture solution was incubated at 37 °C for 100 min, followed by the deactivation of Dam at 80 °C for 20 min.

2.3. KF polymerase/Nt. AlwI facilitated PEC sensing of Dam activity

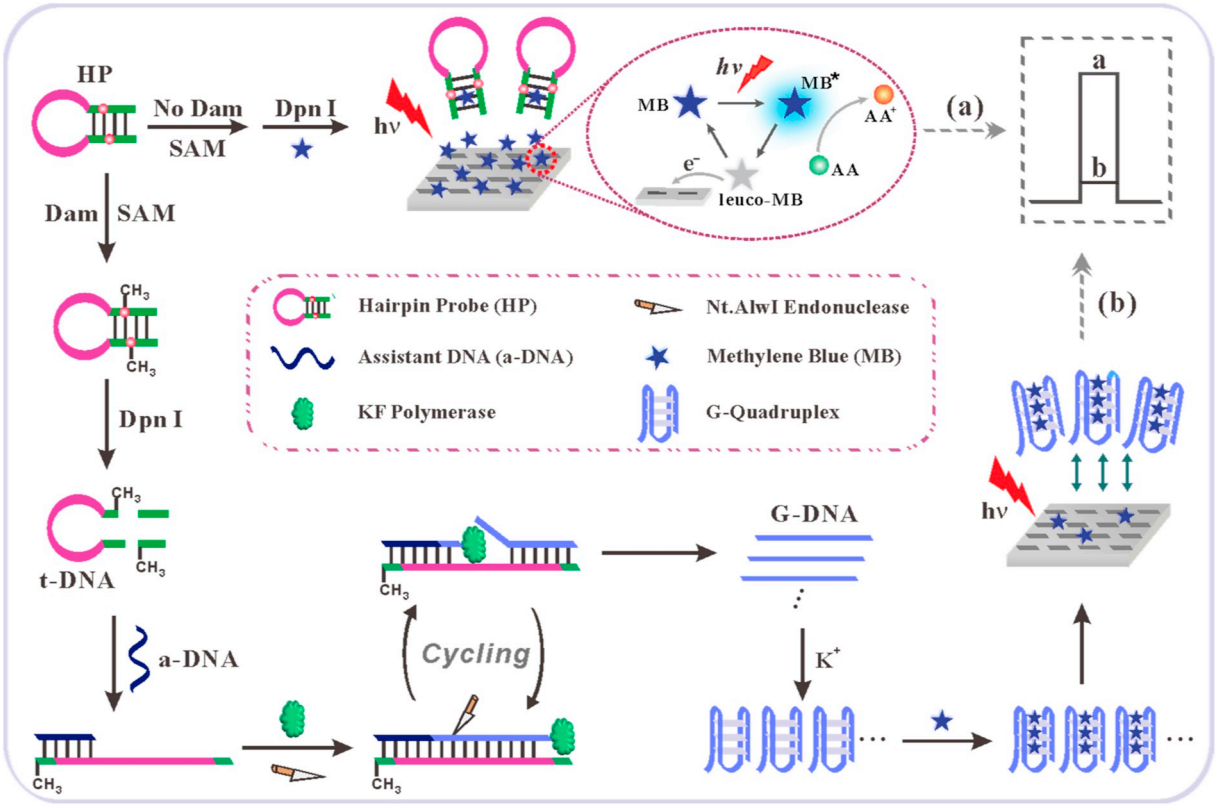
Upon DNA methylation and cleavage, 10 μL of 10 μM a-DNA was added into the above solution and incubated at 37 °C for 60 min. Next, 5 μL of 1600 U/mL KF polymerase in 1 × NEBuffer 2 (pH 7.9, 10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT), 2 μL of 250 μM dNTPs, 5 μL of 1600 U/mL Nt.*Alw*I endonuclease in 1 × NEBuffer 4 (pH 7.9, 50 mM KAc, 20 mM Tris-Ac, 10 mM $Mg(Ac)_2$, 1 mM DTT), and 4 μL of 100 μM MB were added and incubated at 37 °C for 120 min, followed by deactivating the enzymes at 75 °C for 20 min. Finally, 100 μL of 0.2 M AA in phosphate buffer (PB, pH 7.4, 0.1 M Na_2HPO_4, 0.1 M NaH_2PO_4) was added and mixed well, and then PEC responses were detected.

2.4. PEC measurements

All PEC measurements were performed on a Zahner PEC measurement system (ZAHNER-elektrik GmbH & Co. KG, Germany) at room temperature, with a light source of RTR02 light (627 nm) and a constant potential of 0 V (versus Ag/AgCl). A three-electrode system was adopted: a negatively charged bare ITO electrode as the working electrode, an Ag/AgCl electrode as the reference electrode, and a platinum wire as the auxiliary electrode. For the assay of Dam activity in human serum samples, Dam solutions with known concentrations were first spiked into human serum solutions diluted for 100 times by Tris-HCl buffer, then followed by the aforementioned procedures.

2.5. Dam activity inhibition by drugs

For Dam activity inhibition assay, ampicillin and gentamycin were selected as the inhibitors, and the experimental procedures were similar to those mentioned above, except that in the methylation step ampicillin or gentamycin was first added to the solution containing HP, Dam and SAM, and the resulting solution was incubated at 37 °C for 60 min. Next, *Dpn*I was added and the other procedures mentioned above were then carried out, and finally the photocurrents were measured.



Scheme 1. Principle of the label-free and immobilization-free PEC strategy for Dam activity assay: (a) in the absence and (b) in the presence of Dam.

2.6. Nondenaturing polyacrylamide gel electrophoresis (PAGE)

Mixed with 6 × loading buffer, different test solutions were loaded on the 12% nondenaturing polyacrylamide gel. PAGE experiments were performed on a Bio-Rad electrophoresis system (Bio-Rad, USA), in 1 × TBE buffer (pH 7.9, 9 mM Tris-HCl, 9 mM boric acid, 0.2 mM EDTA) at 110 V for 60 min at room temperature. After GelRed staining, the gel was illuminated by UV light and photographed with a Gel Doc XR + Imaging System (Bio-Rad, USA).

3. Results and discussion

3.1. Principle of the PEC assay for Dam activity

The design principle of the label-free and immobilization-free PEC sensing strategy for DNA MTase activity assay is schematically illustrated in Scheme 1, in which Dam is chosen as the model DNA MTase. A hairpin DNA probe (denoted as HP) was designed, which can be recognized by Dam to yield the methylated sequence (5′-G-mA-T-C-3′) *via* the transfer of a methyl group from SAM to the N6-adenine (Barras and Marinus, 1989). In the absence of Dam, the unmethylated HP resists the cleavage by *Dpn*I, thus maintaining its hairpin conformation. With MB added to the system, only a small amount of MB molecules bind to HP *via* the electrostatic interaction and the intercalation into the double helix of the HP short stem (Pan et al., 2007), and most MB molecules are free in solution, being able to diffuse to the surface of the bare ITO photoelectrode. Thus, upon the visible light (627 nm) irradiation, MB is excited to the excited state (MB*) and then transforms to leuco-MB in the presence of AA, which is oxidized to AA^+; subsequently, with leuco-MB changing back to MB, electrical current is generated (Cooper et al., 1999; Hao et al., 2016), resulting in a relatively large photocurrent (Scheme 1a).

Whereas, in the presence of Dam, HP is methylated at the adenine base in Dam recognition sequence to form the methylated sequence (5′-G-mA-T-C-3′), which is then cleaved by *Dpn*I, releasing the trigger DNA (denoted as t-DNA) originally embedded in HP. Next, the assistant single-stranded DNA (denoted as a-DNA) hybridizes with t-DNA, and extends along t-DNA under the catalysis of KF polymerase to form elongated duplex DNA. Subsequently, Nt.*Alw*I endonuclease recognizes the newly formed nicking site and cleave the upper strand of the duplex DNA. The cleaved strand then extend again along the polymerization template t-DNA, to release a guanine-rich DNA strand (denoted as G-DNA) *via* strand displacement. The aforementioned elongation, nicking and strand displacement repeat and result in the formation of a large number of G-DNAs, which fold into G-quadruplexes with the assistance of K^+. Upon intercalation into G-quadruplexes, MB molecules are "frozen" in G-quadruplexes, with their diffusivity significantly reduced, mainly due to the strong interaction between MB and guanines in G-quadruplexes (Ortiz et al., 2011; Zhang et al., 2014). Moreover, due to the electrostatic repulsion between the both negatively charged G-quadruplexes and ITO photoelectrode surface, the intercalated MB molecules are further prevented from reaching the photoelectrode surface. As a result, a significantly decreased PEC response is obtained (Scheme 1b) as compared to that in the absence of Dam (Scheme 1a). Therefore, *via* this "signal-off" fashion, label-free and immobilization-free PEC sensing of Dam activity is readily realized, which possesses the advantages of simplicity, high sensitivity and good specificity.

3.2. Feasibility investigation

To verify the rationality of this label-free and immobilization-free PEC strategy for Dam activity assay, PEC measurements under different conditions were carried out. As shown in Fig. 1A, in the presence of HP, a-DNA and MB, a photocurrent of ca. 305 nA was detected (curve b). Upon the addition of Dam, SAM and *Dpn*I, a slightly increased photocurrent (ca. 316 nA) was observed (curve a), indicating the methylation of HP and the subsequent cleavage by *Dpn*I, which caused the destruction of the hairpin structure of HP, and the presence of more free

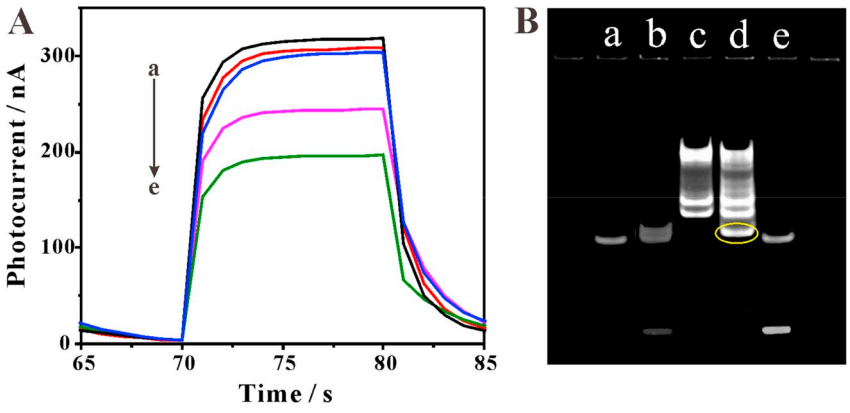


Fig. 1. (A) PEC responses under different conditions: (a) HP + a-DNA + Dam + SAM + *Dpn*I + MB, (b) HP + a-DNA + MB, (c) HP + a-DNA + *Dpn*I + KF + dNTPs + Nt.*Alw*I + MB, (d) HP + a-DNA + Dam + SAM + *Dpn*I + KF + dNTPs + MB, (e) HP + a-DNA + Dam + SAM + *Dpn*I + KF + dNTPs + Nt.*Alw*I + MB. Dam concentration was 10 U/mL, and the other experimental conditions were the same as those stated in Experimental Section. (B) Gel electrophoresis image under different conditions. Lane a: HP only; Lane b: HP + a-DNA + Dam + SAM + *Dpn*I; Lane c: HP + a-DNA + Dam + SAM + *Dpn*I + KF + dNTPs; Lane d: HP + a-DNA + Dam + SAM + *Dpn*I + KF + dNTPs + Nt.*Alw*I; Lane e: HP + a-DNA + SAM + *Dpn*I + KF + dNTPs + Nt.*Alw*I. The concentrations of HP and a-DNA were 0.5 μM, Dam, *Dpn*I and KF polymerase were all 250 U/mL, and SAM, dNTPs and Nt.*Alw*I endonuclease were 0.16 mM, 12.5 μM and 100 U/mL, respectively.

MB in solution. Whereas, if Dam and SAM were absent, but *Dpn*I, KF polymerase, dNPTs and Nt.*Alw*I endonuclease were present, a photocurrent of ca. 300 nA resulted (curve c), similar to that in curve a, suggesting HP remained intact. With the further addition of Dam and SAM, a significant decrease of photocurrent down to ca. 195 nA was observed (curve e), indicating the decline of free MB in solution, due to the intercalation of MB into G-quardruplexes, as demonstrated in Scheme 1b. To further verify the signal amplification achieved *via* the formation of multiple G-quardruplexes, the photocurrent of the reaction system without Nt.*Alw*I was also measured, which was ca. 240 nA (curve d), smaller than that in curve c, but bigger than that in curve e, indicating the occurrence of the DNA methylation, the cleavage by *Dpn*I, and the DNA elongation catalyzed by KF polymerase, which resulted in the formation of duplex DNA and the subsequent intercalation of MB. However, due to the absence of Nt.*Alw*I, the cycling formation of G-rich DNA strands did not take place, and due to the absence of G-quadruplexes, the photocurrent decrease was not as significant as that in curve e.

Next, nondenaturing PAGE analysis was performed to verify the nucleic acid reactions proposed in this sensing strategy, with the results shown in Fig. 1B. As compared to the case where only HP was present (Lane a), with the addition of a-DNA, Dam, SAM and *Dpn*I, two new bands appeared (Lane b): the one above the HP band corresponds to the hybridization product between a-DNA and t-DNA, and the other one below the HP band could be residue a-DNA. Whereas, with the further addition of KF polymerase and dNTPs, a distinctive set of new bands were observed (Lane c), indicating the successful elongation of a-DNA to result in the products with different extent of polymerization. If Nt.*Alw*I was further added, a new band appeared, as circled out in the image (Lane d), indicating the formation of G-DNAs. However, if only Dam is absent in the reaction system, two bands were observed (Lane e), corresponding to HP and a-DNA, which evidently demonstrated that without Dam, HP remained its hairpin structure, and the subsequence HP cleavage, polymerization, and Nt.*Alw*I-assisted nicking did not occur. Therefore, both the PEC and the PAGE results clearly verified the feasibility of this PEC biosensing strategy for Dam activity assay.

Moreover, in the as-proposed PEC strategy, MB in homogeneous solution is used as the photoactive probe, thus its photostability is of importance and was further investigated. As shown in Fig. S1 (in Supporting Information), during the 30 cycles of on/off illumination lasting for 600 s, the photocurrents of MB in homogeneous solution repeated with little variation, especially for the first 15 cycles, indicating good stability of MB upon photo-irradiation during the PEC measurements.

3.3. Analytical performance of PEC Dam activity assay

To ensure the best analytical performance, various experimental conditions were optimized, including the amount of the enzymes, the reaction times, as well as the MB concentration. As shown in Fig. S1 (in Supporting Information), 4 U of *Dpn*I endonuclease, 8 U of KF polymerase and 8 U of Nt.*Alw*I endonuclease were determined to be the optimal amount of the enzymes. 100 min, 60 min and 120 min were selected as the optimal reaction times for DNA methylation, the hybridization between a-DNA and t-DNA, and the KF extension and Nt.*Alw*I nicking, respectively. The optimal concentration of MB was determined to be 2.0 μM.

Next, under the optimum experimental conditions, the analytical performance of the as-proposed PEC sensing strategy was studied by varying the Dam concentration. As shown in Fig. 2A, the photocurrents decreased as the Dam concentration increased from 0 to 50 U/mL, and the photocurrent change ($\Delta I = I_0 - I$, where I_0 and I are the photocurrents in the absence and presence of Dam, respectively) increased with the elevation of Dam concentration (Fig. 2B). Moreover, the photocurrent change exhibits a good linear correlation with the

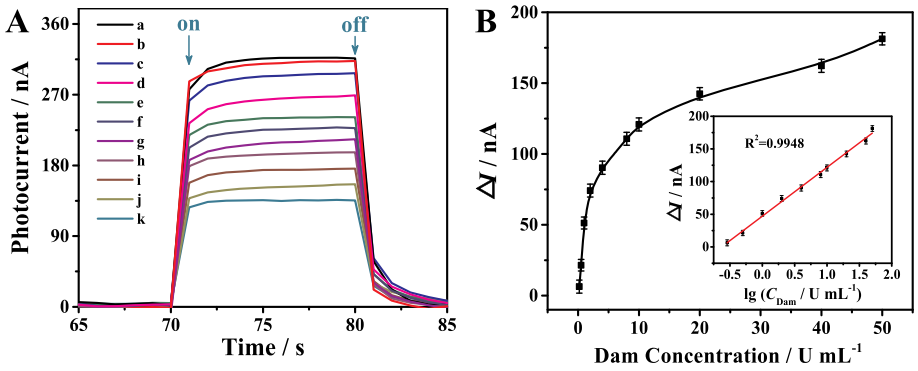


Fig. 2. (A) PEC responses in the presence of Dam with different concentrations: (a) to (k): 0, 0.2, 0.5, 1, 2, 4, 8, 10, 20, 40, and 50 U/mL (from top to bottom); (B) The photocurrent change versus the Dam concentration (from 0.2 to 50 U/mL). Inset: The linear relationship between photocurrent change and the logarithm of Dam concentration ranging from 0.2 to 50 U/mL. The error bars represent the standard deviation of five parallel measurements.

logarithm (to base 10, lg) of the Dam concentration from 0.2 to 50 U/mL (inset of Fig. 2B). The regression equation is $\Delta I = 46.99 + 74.91 \lg C_{Dam}$, with a coefficient of determination of $R^2 = 0.9948$, where ΔI is the photocurrent change (nA), and C_{Dam} stands for the Dam concentration (U/mL). The detection limit for Dam activity assay was 0.06 U/mL (based on S/N of 3), which is comparable or superior to most of the DNA MTase sensing approaches previously reported in literature (Table S1 in Supporting Information). As compared to those approaches with comparable detection limits, the as-proposed PEC strategy exhibited the advantage of simplicity, making it a competitive candidate for DNA MTase activity assay. In addition, the repeatability of the PEC sensing strategy was tested. The relative standard deviation (RSD) of five repeated measurements was 3.45% for 1 U/mL Dam and 3.26% for 10 U/mL Dam, demonstrating acceptable repeatability of the PEC assay for Dam activity.

Furthermore, to evaluate the specificity of this PEC strategy for Dam activity assay, CpG Methyltransferase (M.SssI), GpC Methyltransferase (M.CviPI), and *Alu*I MTase were selected as the potential interfering MTases (Zhou et al., 2017), and PEC measurements were performed by substituting Dam with these interfering MTases, respectively. As clearly illustrated in Fig. 3, compared with the condition where neither Dam nor any of the interfering MTases was present (denoted as Control), obvious photocurrent decrease was observed for 20 U/mL Dam, whereas, the photocurrent for each of the interfering MTases (20 U/mL), *i.e.* M.SssI, M.CviPI and *Alu*I, was almost the same as that of Control, due to the facts that neither of the interfering MTases can methylate the Dam recognition sequences (5′-GATC-3′) in HP, and thus the unmethylated HP substrates resist the cleavage by *Dpn*I, preventing the subsequent DNA elongation and Nt.*Alw*I endonuclease-catalyzed nicking. This proved the good specificity of this PEC sensing strategy toward Dam activity detection. Therefore, the aforementioned results verified that the label-free and immobilization-free PEC approach we proposed here can be adopted for highly sensitive Dam activity assay with acceptable precision, good repeatability and specificity.

3.4. Quantification of Dam in human serum samples

To validate the applicability of this PEC strategy for Dam activity detection in real samples, the PEC responses with Dam spiked in human serum samples were measured and compared with those in the absence of human serum. As illustrated in Fig. S2 (in Supporting Information), in the control experiments (*i.e.* without Dam), relative big photocurrents were obtained for buffer and human serum samples, with almost the same amplitude. In the cases where Dam was present, the photocurrents decreased accordingly both in buffer and in human serum samples, suggesting the success of detecting Dam activity in human serum samples. Furthermore, the accuracy of the PEC assay for

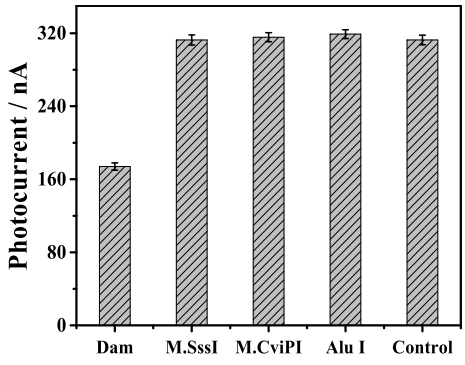


Fig. 3. Photocurrent comparison in the presence of different MTases (20 U/mL), in which Control depicts the condition in the absence of MTases. The error bars represent the standard deviation of five parallel measurements, with the RSDs of 2.30%, 1.76%, 1.58%, 1.50% and 1.70%, respectively.

Table 1
Detection results of Dam spiked in human serum samples.

Dam spiked (U/mL)	Dam detected (U/mL) (n = 5)	mean recovery[a] (%)	RSD[b] (%)
0.0	Not detected	/	/
1.0	0.95 ± 0.02	95.4	2.10
10.0	9.95 ± 0.35	99.5	3.50
20.0	20.30 ± 0.83	101.5	4.08

[a] Recovery = $(C_{detected}/C_{spiked}) \times 100\%$.
[b] RSD stands for the relative standard deviation.

real sample detection was tested, by carrying out PEC measurements on human serum samples (diluted 100 times) spiked with Dam solutions with different concentrations (0, 1.0, 10.0 and 20.0 U/mL). As shown in Table 1, the average recovery was in the range of 95.4%–101.5%, with acceptable RSD no bigger than 4.08%. These results indicate that the as-proposed PEC sensing strategy can potentially be applied in accurate detection of Dam activity in real samples such as human serum.

3.5. Evaluation of Dam activity inhibition

Since Dam plays a vital role in DNA mismatch repair, as well as the regulation of replication and protein expression, it is of great significance to evaluate and screen Dam inhibitors. Thus the capability of the as-proposed strategy in evaluating Dam activity inhibition was further investigated by selecting two antibiotics (ampicillin and gentamycin) as the model inhibitors, which are well-known broad-spectrum antibiotics, and have been adopted as inhibitors of methyltransferase (Elsinghorst, 1994; Rauf et al., 2017; Trafford et al., 1962). The study of inhibitory effect of these antibiotics on Dam activity may provide a valuable approach for antibacterial therapy. As presented in Fig. 4A, in the absence of inhibitors, a relatively large photocurrent change (ΔI, the photocurrent difference in the absence and presence of Dam) was obtained, suggesting the occurrence of DNA methylation; however, in the presence of either ampicillin (20 μM) or gentamycin (2 μM), smaller photocurrent changes (ΔI) were observed, indicating the inhibition of Dam activity. Of the two inhibitors, gentamycin exhibits a higher inhibitory effect on Dam activity, corresponding well to the results reported by Li and co-workers (Rauf et al., 2017). In addition, the inhibitory effects of gentamycin with different concentrations were investigated, and the relationship between the relative activity and the gentamycin concentration is shown in Fig. 4B, in which the relative activity was calculated as follows:

$$\text{Relative activity} = \frac{I_0 - I_i}{I_0 - I_d}$$

where I_0, I_d and I_i were the photocurrents without Dam, with Dam only, and with both Dam and inhibitor (gentamycin), respectively. With the gentamycin concentration increased from 0 to 6 μM, the Dam activity decreased accordingly, with the relative activity reduced from 1.0 to around 0.35. The IC_{50} value, the dose of inhibitor needed to inhibit the enzyme activity by 50%, was then determined to be around 3.0 μM for gentamycin. Therefore, these results evidently proved that the as-proposed PEC approach can be used for Dam activity inhibition study and inhibitor screening.

4. Conclusions

In conclusion, we have developed a novel label-free and immobilization-free PEC biosensing strategy, in which MB in homogeneous solution is adopted as the PEC signal probe and negatively charged bare ITO as the photoelectrode. Through the diffusivity difference between free MB molecules and MB intercalated in G-quadruplexes, as well as enzyme-aided signal amplification approach, highly sensitive and specific PEC sensing assay of the activity of a proof-of-

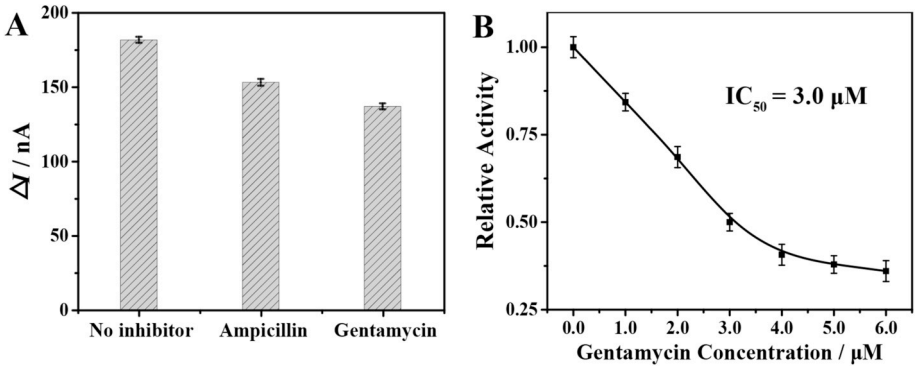


Fig. 4. (A) Inhibitory effects of ampicillin (20 μM) and gentamycin (2 μM) on Dam activity. (B) Effects of Dam activity inhibition by gentamycin with different concentrations. The concentration of Dam was 50 U/mL, and the error bars represent the standard deviation of five parallel measurements.

concept MTase (Dam) has been achieved, with a detection limit of 0.06 U/mL, comparable or superior to most of those previously reported in literature, and the ability to distinguish Dam from the interfering MTase. Moreover, the as-proposed strategy shows good applicability in detecting the activity of Dam spiked in human serum samples, and the inhibition effects of antibiotics on Dam activity have also been evaluated. Therefore, this label-free and immobilization-free PEC biosensing strategy demonstrates excellent analytical performance for the assay of Dam activity and inhibition, and exhibits additional merits of simplicity and good repeatability due to the elimination of both labelling and immobilization procedures, making this approach a promising candidate for the application in highly sensitive, facile and reliable bioanalysis and drug screening.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CRediT authorship contribution statement

Ting Hou: Conceptualization, Project administration, Software, Supervision, Resources, Writing - review & editing. **Ningning Xu:** Conceptualization, Data curation, Investigation, Methodology, Writing - original draft. **Wenxiao Wang:** Conceptualization, Data curation, Investigation, Methodology. **Lei Ge:** Conceptualization, Investigation, Methodology. **Feng Li:** Funding acquisition, Project administration, Resources.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.111395.

References

Barras, F., Marinus, M.G., 1989. Trends Genet. 5, 139–143.
Bergerat, A., Guschlbauer, W., Fazakerley, G.V., 1991. Proc. Natl. Acad. Sci. U.S.A. 88 (15), 6394–6397.
Boye, E., Marinus, M.G., Løbner-Olesen, A., 1992. J. Bacteriol. 174 (5), 1682–1685.
Chen, S., Lv, Y., Shen, Y., Ji, J., Zhou, Q., Liu, S., Zhang, Y., 2018. ACS Appl. Mater. Interfaces 10 (8), 6887–6894.
Cooper, J.A., Woodhouse, K.E., Chippindale, A.M., Compton, R.G., 1999. Electroanalysis 11 (17), 1259–1265.
Cooper, J.A., Wu, M., Compton, R.G., 1998. Anal. Chem. 70 (14), 2922–2927.
Elsinghorst, E.A., 1994. Methods Enzymol. 236, 405–420.
Ge, L., Wang, W.X., Hou, T., Li, F., 2016a. Biosens. Bioelectron. 77, 220–226.
Ge, L., Wang, W.X., Sun, X.M., Hou, T., Li, F., 2016b. Anal. Chem. 88 (4), 2212–2219.
Hao, Q., Shan, X.N., Lei, J.P., Zang, Y., Yang, Q.H., Ju, H.X., 2016. Chem. Sci. 7 (1), 774–780.
Hong, Q., Ge, L., Wang, W.X., Liu, X.J., Li, F., 2018. Biosens. Bioelectron. 121, 90–95.
Hou, T., Li, W., Liu, X., Li, F., 2015. Anal. Chem. 87 (22), 11368–11374.
Hou, T., Xu, N.N., Wang, W.X., Ge, L., Li, F., 2018. Anal. Chem. 90 (15), 9591–9597.
Hou, T., Zhang, L.F., Sun, X.Z., Li, F., 2016. Biosens. Bioelectron. 75, 359–364.
Lennard, L., Singleton, H.J., 1994. J. Chromatogr. B Biomed. Sci. Appl. 661 (1), 25–33.
Li, W., Liu, X.J., Hou, T., Li, H.Y., Li, F., 2015. Biosens. Bioelectron. 70 (0), 304–309.
Lin, Y., Zhou, Q., Tang, D., 2017a. Anal. Chem. 89 (21), 11803–11810.
Lin, Y., Zhou, Q., Tang, D., Niessner, R., Knopp, D., 2017b. Anal. Chem. 89 (10), 5637–5645.
Liu, X.J., Li, W., Hou, T., Dong, S.S., Yu, G.H., Li, F., 2015. Anal. Chem. 87 (7), 4030–4036.
Lubin, A.A., Vander Stoep Hunt, B., White, R.J., Plaxco, K.W., 2009. Anal. Chem. 81 (6), 2150–2158.
Muren, N.B., Barton, J.K., 2013. J. Am. Chem. Soc. 135 (44), 16632–16640.
Ortiz, M., Fragoso, A., Ortiz, P.J., O'Sullivan, C.K., 2011. J. Photochem. Photobiol., A 218 (1), 26–32.
Osterloh, F.E., 2013. Chem. Soc. Rev. 42 (6), 2294–2320.
Pan, D., Zuo, X., Wan, Y., Wang, L., Zhang, J., Song, S., Fan, C., 2007. Sensors 7 (11).
Rauf, S., Zhang, L., Ali, A., Ahmad, J., Liu, Y., Li, J.H., 2017. Anal. Chem. 89 (24), 13252–13260.
Robertson, K.D., 2005. Nat. Rev. Genet. 6 (8), 597–610.
Robertson, K.D., Wolffe, A.P., 2000. Nat. Rev. Genet. 1 (1), 11–19.
Shen, Q.M., Han, L., Fan, G.C., Abdel-Halim, E.S., Jiang, L.P., Zhu, J.J., 2015. Biosens. Bioelectron. 64, 449–455.
Su, F.X., Wang, L.M., Sun, Y.Y., Liu, C.H., Duan, X.R., Li, Z.P., 2015. Chem. Commun. 51 (16), 3371–3374.
Sun, Y., Sun, Y., Tian, W., Liu, C., Gao, K., Li, Z., 2018. Chem. Sci. 9 (5), 1344–1351.
Tan, Y., Wei, X.F., Zhao, M.M., Qiu, B., Guo, L.H., Lin, Z.Y., Yang, H.H., 2015. Anal. Chem. 87 (18), 9204–9208.
Trafford, J.A.P., Maclaren, D.M., Lillicrap, D.A., Barnes, R.D.S., Houston, J.C., Knox, R., 1962. Lancet 279 (7237), 987–990.
van Steensel, B., Henikoff, S., 2000. Nat. Biotechnol. 18 (4), 424–428.
Wei, X.F., Ma, X.M., Sun, J.J., Lin, Z.Y., Guo, L.H., Qiu, B., Chen, G.N., 2014. Anal. Chem. 86 (7), 3563–3567.
Wu, Y., Zhang, B., Guo, L.H., 2013. Anal. Chem. 85 (14), 6908–6914.
Xuan, F., Luo, X.T., Hsing, I.M., 2012. Anal. Chem. 84 (12), 5216–5220.
Yang, Z.Q., Wang, F.R., Wang, M., Yin, H.S., Ai, S.Y., 2015. Biosens. Bioelectron. 66, 109–114.
Yin, H., Sun, B., Zhou, Y., Wang, M., Xu, Z., Fu, Z., Ai, S., 2014. Biosens. Bioelectron. 51, 103–108.
Zhang, F.T., Nie, J., Zhang, D.W., Chen, J.T., Zhou, Y.L., Zhang, X.X., 2014. Anal. Chem. 86 (19), 9489–9495.
Zhang, K., Lv, S., Lu, M., Tang, D., 2018. Biosens. Bioelectron. 117, 590–596.
Zhao, H.Y., Wang, L., Jiang, W., 2016. Chem. Commun. 52 (12), 2517–2520.
Zhao, W.W., Xu, J.J., Chen, H.Y., 2014. Chem. Rev. 114 (15), 7421–7441.
Zhao, W.W., Xu, J.J., Chen, H.Y., 2015. Chem. Soc. Rev. 44 (3), 729–741.
Zhao, W.W., Xu, J.J., Chen, H.Y., 2018. Anal. Chem. 90 (1), 615–627.
Zhou, X., Zhao, M., Duan, X., Guo, B., Cheng, W., Ding, S., Ju, H., 2017. ACS Appl. Mater. Interfaces 9 (46), 40087–40093.