Biosensors and Bioelectronics 54 (2014) 565–570

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A methylation-blocked cascade amplification strategy for label-free colorimetric detection of DNA methyltransferase activity ☆

Yongxi Zhao [a,b,*], Feng Chen [a], Manli Lin [a], Chunhai Fan [b,*]

[a] Key Laboratory of Biomedical Information Engineering of Education Ministry, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, Shaanxi 710049, PR China

[b] Division of Physical Biology, and Bioimaging Center, Shanghai Synchrotron Radiation Facility, CAS Key Laboraotory of Interfacial Physics and Technology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, PR China

ARTICLE INFO

Article history:
Received 28 September 2013
Received in revised form
17 November 2013
Accepted 18 November 2013
Available online 26 November 2013

Keywords:
DNA methyltransferase
Methylation-blocked cascade amplification
DNAzyme
Label-free colorimetric detection
Inhibitor

ABSTRACT

DNA methyltransferase (MTase), catalyzing DNA methylation in both eukaryotes and prokaryotes, is closely related with cancer and bacterial diseases. Although there are various methods focusing on DNA MTase detection, most of them share common defects such as complicated setup, laborious operation and requirement of expensive analytical instruments. In this work, a simple strategy based on methylation-blocked cascade amplification is developed for label-free colorimetric assay of MTase activity. When DNA adenine methylation (Dam) MTase is introduced, the hairpin probe is methylated. This blocks the amplified generation of G-riched DNAzyme by nicking endonuclease and DNA polymerase, and inhibits the DNAzyme-catalyzed colorimetric reaction. Contrarily, an effective colorimetric reaction is initiated and high color signal is clearly observed by the naked eye in the absence of Dam MTase. A satisfying sensitivity and high selectivity are readily achieved within a short assay time of 77 min, which are superior to those of some existing approaches. Additionally, the application of the sensing system in human serum is successfully verified with good recovery and reproducibility, indicating great potential for the practicality in high concentrations of interfering species. By using several anticancer and antimicrobial drugs as model, the inhibition of Dam MTase is well investigated. Therefore, the proposed method is not only promising and convenient in visualized analysis of MTase, but also useful for further application in fundamental biological research, early clinical diagnosis and drug discovery.

1. Introduction

DNA methylation, a widespread epigenetic modification in both prokaryotes and eukaryotes, plays a vital role in lots of biological processes by regulating gene expression (Reisenauer et al., 1999; Robertson, 2005; Szyf, 2003, 2005). The DNA methylation process is carried out by DNA methyltransferase (MTase) which catalyzes the covalent addition of a methyl group to target adenine or cytosine residues in the recognition sequences (Cheng and Roberts, 2001). In recent years, more and more studies have confirmed that aberrant DNA methylation could be regarded as a new cancer biomarker (Esteller, 2007; Robertson, 2005; Szyf, 2003, 2005). Also, abnormal level of DNA MTase has profound implications in some critical diseases such as cancer and bacterial disease (Brueckner and Lyko, 2004; Erova et al., 2006; Garcia-Del Portillo et al., 1999; Robertson, 2005; Toyota et al., 1999; Tsankova et al., 2007). Thus, DNA MTase has been treated as a potential target in anticancer therapy and for new antimicrobial drugs, and the estimate of DNA MTase activity together with screening of its inhibitors has aroused an increasing interest over the past several decades.

So far, some traditional assays, using radioactive labeling, gel electrophoresis and high performance liquid chromatography for the estimation of DNA MTase activity, have been well-established (Adams et al., 1991; Bergerat et al., 1991; Boye et al., 1992; Messer and Noyer-Weidner, 1988). Due to the intrinsic drawbacks, including DNA-consuming, labor-intensive, radiolabeled substrates required or of insufficient sensitivity, these methods have rather limited application. Recently, several fascinating strategies have been developed to overcome the above shortcomings. These techniques include fluorescence assay (Lee et al., 2011; Li et al., 2007; Tian et al., 2012; Zhao et al., 2013), bioluminescence detection (Jiang et al., 2012), electrochemical biosensor (Li et al., 2012a; Su et al., 2012; Wang et al., (2013); Wu et al., 2012; Yin et al., 2013), chemiluminescence (Bi et al., 2013; Zeng et al., 2013) and electrogenerated chemiluminescence

☆The authors declare no competing financial interest.

* Corresponding author. Tel./fax: +862 982 663 454.
E-mail addresses: yxzhao@mail.xjtu.edu.cn (Y. Zhao), fchh@sinap.ac.cn (C. Fan).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.11.055

method (Li et al., 2012b, 2013). All the above approaches indeed improve the sensing performance and offer a high sensitivity; however, they demand complicated processes and complex or/and expensive analytical instruments. What is more, some of them may require trained personnel to operate related instruments. Contrarily, colorimetric strategy is extremely attractive owing to its rapidity, simplicity, cost-effectiveness. More importantly, it may be easily read out by the naked eye without requiring expensive analytical instruments, which are immensely significant in point-of-care testing. Based on the DNA-modified gold nanoparticles (AuNPs), several colorimetric biosensors have been successively developed for DNA MTase activity analysis. For example, Qu' group (Song et al., 2009) and Li' group (Liu et al., 2009) proposed DNA MTase assay employing MTase-responsive DNA-AuNPs redispersion and aggregation, respectively. Lately, another colorimetric method using MTase-protection dispersion of DNA-AuNPs has been reported by Jiang et al. (Wu et al., 2013). Thanks to the intense surface plasmon resonance, these AuNPs-based approaches show an excellent colorimetric signal and improve the analytical performance. However, they all need to functionalize the AuNPs with thiol-label DNA, which is to say, the process not only leads to complication and relatively high cost, but also takes several days. In addition, the suspension of aggregated AuNPs is unstable in solution owing to the increasing particle size together with reducing surface repelling force (Guo et al., 2013). As a result, the suspension changes into colorless over time due to forming sedimentation of AuNPs. This unfavorable phenomenon can impede accurate quantitative determination. Besides, the false positive resulting from the nonspecific aggregation of the functionalized AuNPs in complex biological samples would restrict its practical application. Alternatively, Nie et al. proposed a label-free colorimetric biosensor for DNA MTase activity on the basis of methylation-responsive DNAzyme (Li et al., 2010), which avoids the disadvantages of above AuNPs-based methods. The DNAzyme involved is a G-riched single strand DNA, which mimics horseradish peroxidase (HRP) and catalyzes the colorimetric reaction. This DNAzyme-based colorimetric method is out of chemical modification and does not involve the use of nanoparticle. Unfortunately, it must employ other three enzymes and two long DNA strands to perform the reaction, making the design complicated. Since each enzyme is very sensitive to its own buffer, the complex reaction conditions would lead to poor reaction efficiency. Meanwhile, a lengthy assay time about 160 min is taken by this method. Therefore, to develop label-free, colorimetric, simple and rapid methods for DNA MTase detection is still a challenge.

Lately, we have reported a novel fluorescence biosensor for amplified detection of DNA adenine methylation (Dam) MTase by using only one enzyme (Chen and Zhao, 2013). Inspired by this unique work, herein, we developed a simple and label-free colorimetric approach for rapid detection of Dam MTase activity and inhibition based on methylation-blocked cascade amplification. This innovative strategy only involves other two enzymes and one hairpin DNA probe, which is simpler, more efficient and less costly than the previously reported method (Li et al., 2010). Furthermore, the colorimetric assay is fast finished in 77 min, and can be successfully carried out in complex matrixes such as human serum sample.

2. Experimental section

2.1. Reagents and materials

Dam MTase, Nt.AlwI and S-adenosylmethionine (SAM) were obtained from New England Biolabs Inc. (Beijing, China). Klenow Fragment (3′→5′ exo$^-$), dNTPs, hemin, 2,2′-azino-bis(3-ethylbenzothiazoline)-6-sulfonate disodium salt (ABTS), H_2O_2, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonate (HEPES), gentamycin, 5-fluorouracil and benzyl penicillin was purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). All of the other chemicals were of analytical reagent grade and used without further purification. The ultrapure water with an electrical resistance larger than 18.2 MΩ was obtained through a Milli-Q system (Millipore, Bedford, MA). All oligonucleotides used in this study were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China) and sequences were as follows:

hairpin DNA probe 1

5′-TCCCAACCCGCCCTACCCTGAT**GATCC**ATAC*TTTT*GTAT**GGATC**ATCAGGGTAGGGCGGGTTGGGA-3′

hairpin DNA probe 2

5′-TCCCAACCCGCCCTA CCCTGAT**GATCC**ATAC*TTTT*GTAT**GGATC**ATCA-3′

G-riched DNAzyme

5′-GGGTAGGGCGGGTTGGGA-3′

The bold letters represent the recognition sequence for Nt.AlwI, and the DNAzyme sequence is highlighted in green. The italic poly-T is the loop of the hairpin probe.

2.2. Absorbance measurements

Absorbance measurements were performed using a T60 UV−Vis spectrophotometer (Beijing Purkinje General Instrument Co, Ltd., China). The absorption spectra of the solution were measured in the wavelength ranging from 500 to 400 nm.

2.3. Colorimetric assay of Dam MTase activity

Prior to use, the hairpin probe (1 μM) in the annealing buffer (10 mM Tris–HCl, 50 mM NaCl, 1 mM EDTA, pH=7.8) was heated to 95 °C for 2 min and gradually cooled down to 45 °C for another 10 min. The NEBuffer 2 (10 mM Tris–HCl, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH=7.9) was used as reaction buffer.

In a typical experiment, 20 μL of this buffer containing 2 μL of the hairpin probe, 160 μM SAM and different target concentrations was incubated at 37 °C for 30 min firstly. Then, 5 U of Nt.AlwI, 2.5 U of Klenow Fragment polymerase and 4 μL of 2.5 mM dNTPs were introduced into the resulting mixture. At last, the mixture was 40 μL in a total volume, and was allowed to proceed at 37 °C for another 30 min. After incubation, the solution was diluted to 160 μL with 95 μL of H_2O, 20 μL of HEPES buffer (250 mM HEPES, 100 mM KCl, 2 M NaCl, 0.5% TritonX-100, pH=6.2) and 5 μL of 20 μM hemin. Subsequently, it was incubated at room temperature for 10 min to form the hemin/G-quadruplex DNAzyme structures. Finally, substrates, including 20 μL of ABTS (20 mM) and 20 μL of H_2O_2 (20 mM), were added in the obtained mixture, respectively. The biocatalyzed oxidation of $ABTS^{2-}$ is immediately initiated and the absorption spectra were recorded in 7 min by UV−Vis spectrometer.

2.4. Gel electrophoresis

Gel electrophoresis was used to investigate the generation of G-riched DNAzyme sequence, confirming the feasibility of the sensing system. Samples for gel electrophoresis assays were prepared as follows: (1) hairpin DNA probe (0.5 μM) only; (2) G-riched DNAzyme (5 μM) only; (3) the sample contained hairpin DNA probe (0.5 μM) and SAM (160 μM) was incubated at 37 °C for 60 min first. Then, Nt.AlwI (20 U), Klenow Fragment (10 U) and dNTPs (0.25 mM) was added to carried out for another 60 min; (4) the sample was prepared similarly as (3) except with Dam MTase (80 U/mL). The samples were put on a 20% polyacrylamide

gel to separate the related products from the substrate. The gel electrophoresis experiments were carried in 1 × Tris-Borate-EDTA (TBE) buffer (90 mM Tris, 90 mM boric acid, 10 mM EDTA, pH 8.0) by using the Mini-PROTEAN Tetra Cell system (Bio-Rad Labs., Richmond, CA). They were performed at the constant voltage of 45 V for 2.5 h. After silver-stained, the gels were imaged with a Canon digital camera.

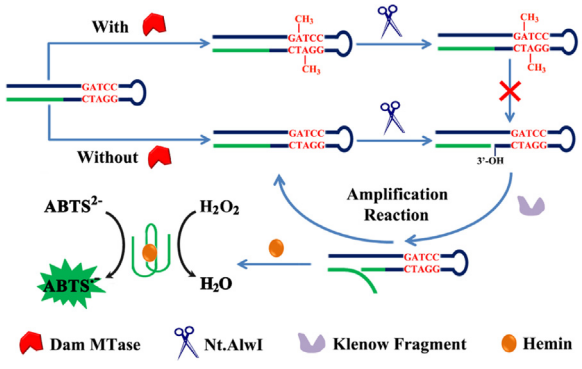
2.5. Dam MTase inhibition evaluation

To investigate whether inhibitors (gentamycin, 5-fluorouracil and benzyl penicillin) have influence on Nt.AlwI and Klenow Fragment polymerase, a control experiment was carried out. Before the addition of 5 U of Nt.AlwI and 2.5 U of Klenow Fragment polymerase, inhibitors were introduced into 40 μL reaction mixture similar to that of Dam MTase detection without Dam MTase. The solution was incubated at 37 °C for 30 min, then the colorimetric reaction was carried out as mentioned above. For Dam MTase inhibition experiments, the procedure was similar to the detection of its activity that noted above, except that different concentrations of inhibitors were added together with Dam MTase.

3. Results and discussion

3.1. Design of methylation-blocked cascade amplification based label-free colorimetric biosensor

As illustrated in Scheme 1, only one DNA strand is involved in our detection system. This hairpin DNA probe is particularly designed to contain two functional domains. The green one is the sequence of G-riched DNAzyme. And another one highlighted in red represents the recognition site (5′-GGATC-3′) for Nt.AlwI, a nicking endonuclease (NEase) which can recognize dsDNA sequence but cuts only one strand. Besides Nt.AlwI, the sensing system also contains a DNA polymerase (Klenow Fragment). In the absence of target, Nt.AlwI will be bound to the hairpin DNA probe on its recognition site, and nick the dsDNA to generate a 3′-OH end. This 3′–OH terminal is presented to an engaging primer which triggers the DNA polymerization reaction catalyzed by DNA polymerase in the presence of dNTPs (act as fuel). The Klenow Fragment polymerase will also displace the original DNAzyme sequence during synthesis of new DNAzyme sequence. Simultaneously, the NEase can scissor the just synthesized hairpin DNA probe, resulting in the generation of new active site for next polymerization reaction and the concomitant displacement of already synthesized DNAzyme strand. Through such amplification reaction including scission, polymerization and displacement three processes, thousands of DNAzyme strands can be produced. Subsequently, by adding the hemin, these G-riched strands fold into the hemin/G-quadruplex DNAzyme structures, activating the HRP-mimicking catalytic activity. Eventually, the colorimetric reaction of oxidating $ABTS^{2-}$ to the green product $ABTS^{\bullet-}$ is performed by these DNAzymes with the help of H_2O_2. The cascade amplification signal would improve the assay sensitivity well. On the contrary, when Dam MTase is introduced into the system, the hairpin DNA probe will be methylated. Since Nt.AlwI is sensitive to the methylation catalyzed by Dam MTase, the cleavage reaction of hairpin DNA probe is inhibited by the Dam MTase-catalyzed methylation. And the DNA polymerase is unable to carry out the polymerization and displacement processes in the absence of 3′-OH end. As a result, the hairpin DNA probe maintains in an "inactivated" conformation and no DNAzyme strands are generated. Ultimately, the cascade colorimetric reaction is blocked by Dam MTase-catalyzed methylation. With the higher concentration of Dam MTase, the blocking effect is more pronounced. In this way, by the naked eye or with simple UV–Vis spectroscopy, we could easily detect the target without any complex chemical modification of DNA or nanoparticle.



Scheme 1. Schematic illustration of methylation-blocked cascade amplification based label-free colorimetric biosensor for Dam MTase activity assay.

3.2. Feasibility of the assay

To verify the feasibility of the proposed strategy, several experiments were performed. As shown in Fig. 1A, the color of the sensing system turned from colorless to green when Dam MTase is absent, indicating that lots of DNAzymes are generated by the amplification reaction. However, it remained colorless in the presence of Dam MTase, demonstrating that the cascade colorimetric reaction was blocked by Dam MTase-catalyzed methylation as described above. This result is also proved by their UV–Vis absorption spectra. It can be seen that the absorbance intensity at 418 nm in the absence of Dam MTase (Fig. 1A, curve a) is about 11-fold higher than that of with Dam MTase (Fig. 1A, curve b). This excellent signal-to-noise ratio may contribute to improve the detection sensitivity. In addition, another experiment using G-riched DNAzyme was tested to demonstrate many cycles of

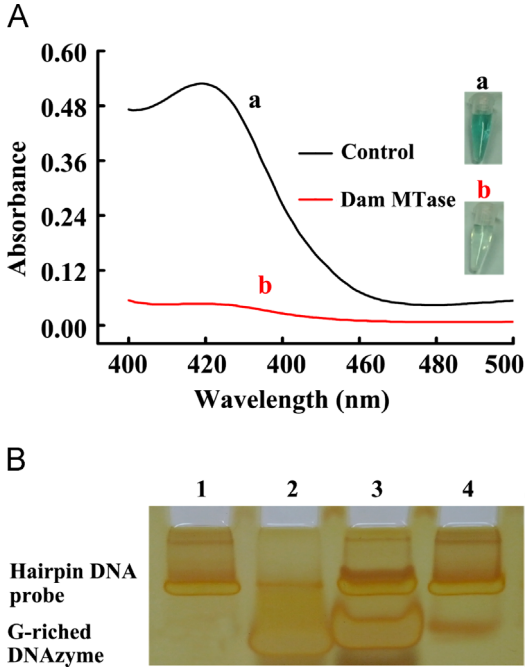


Fig. 1. (A) UV–Vis absorption spectra of the sensing system in the absence (a) and presence (b) of Dam MTase (40 U/mL). The corresponding photographs were also presented, respectively. (B) Electrophoresis analysis: (1) hairpin DNA probe only (0.5 μM); (2) G-riched DNAzyme only (5 μM); (3) without Dam MTase; (4) with Dam MTase (80 U/mL).

DNAzyme generation by the amplification reaction. As shown in Fig. S1, the absorbance value of G-riched DNAzyme is much lower than that of amplification reaction catalyzed by nicking endonuclease and Klenow fragment, indicating the recycling generation of G-riched DNAzyme during the amplification reaction.

Besides, the viability of proposed system is further estimated by gel electrophoresis. The corresponding results were shown in Fig. 1B. A well-defined new band of the G-riched DNAzyme (18 nt) appeared in the absence of Dam MTase (lane 3). In contrast, the band of the G-riched DNAzyme is inconspicuous when it was treated with Dam MTase (lane 4). These results confirm that Dam MTase-catalyzed methylation blocks the amplification reaction triggered by NEase and DNA polymerase. Therefore, the analysis of Dam MTase can be successfully performed by this label-free colorimetric biosensor.

3.3. Optimization of experimental conditions

To achieve the best analytical performance, assay conditions including the structure of hairpin DNA probe, enzyme concentrations and reaction times were optimized. The hairpin DNA probe was designed not only as the substrate for Dam MTase and but also as the template for DNAzyme synthesis. Theoretically, owing to the ability to produce more HRP-mimicking DNAzymes, the hairpin DNA probe containing the DNAzyme sequence (hairpin DNA probe 1) will contribute to higher detection sensitivity than that without this sequence (hairpin DNA probe 2). Thus, the structure of hairpin DNA probe is investigated, and the result is depicted in Fig. S2 in Supplementary material. As expected, the signal of hairpin DNA probe 1 is obviously higher, and it is chosen for the following experiments. To the best of our knowledge, it is the first time that the dsDNA template with the signal sequence for the NEase and polymerase-assisted amplification methods is artificially employed. Compared to the traditional ssDNA template without the signal sequence, the dsDNA template can generate signals during the first circle of the amplification reaction rather than from the second circle. In addition, the unreacted ssDNA template, complementary with the signal strand, will hybridize to the newly generated signal strand, leading to the lower signal response. Oppositely, it can't happen when the dsDNA template is present, resulting in a higher signal. This innovative design may help reach the highest detection signal within a short assay time. It is noteworthy that the previously reported method (Li et al., 2010) used a template without the DNAzyme sequence, which possibly leads to its long amplification reaction time (120 min).

The concentrations of NEase and DNA polymerase are crucial in the sensing system. On one hand, the low concentrations of these two enzymes can adversely affect the amplification efficiency and result in the decrease of DNAzymes. On the other hand, lots of enzymes generate high cost. Therefore, their concentrations should be optimized carefully. As can be seen in Fig. S3, the colorimetric signal is enhanced as the concentration of Nt.AlwI increased, and it reaches a maximum at 5 Unit. As a result, 5 Unit is selected as the optimum concentration. Similarly, 2.5 Unit of the DNA polymerase is used for the following assay.

Additionally, the reaction time is another significant factor that influence the sensing performance. In our system, a long time of amplification reaction is expected to yield DNAzymes of elevated concentration, meaning high colorimetric signal. The investigated results indicate that the absorbance intensity increases with the increase of amplification reaction time (Fig. 2A). And 30 min was employed for the following experiments due to the highest signal. Then, the time of methylation reaction catalyzed by Dam MTase is optimized and the result is presented in Fig. 2B. Considering the balance between detection sensitivity and assay time, the optimized time of methylation reaction is set to be 30 min. Meanwhile,

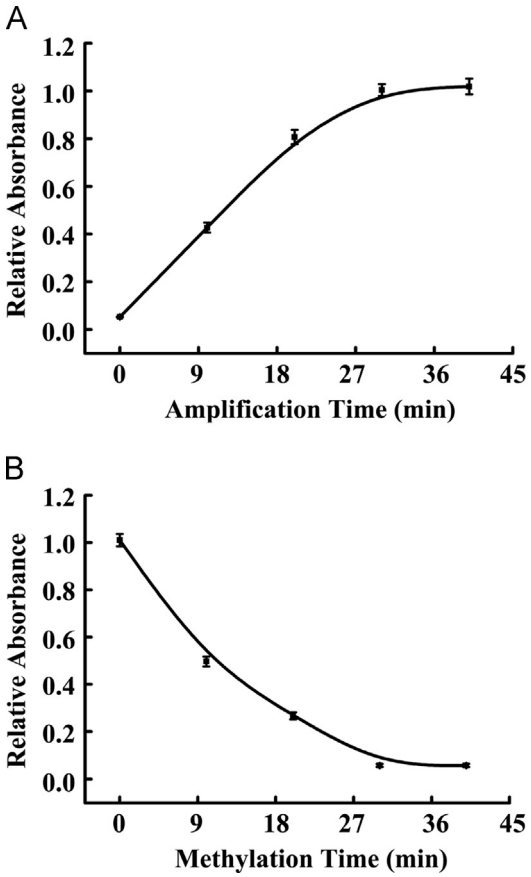


Fig. 2. (A) The amplification time-dependent relative UV–vis absorbance of 418 nm with a fixed methylation time of 30 min. (B) The methylation time-dependent relative UV–vis absorbance of 418 nm with a fixed amplification time of 30 min. The Dam MTase concentration in all figures is 40 U/mL. The error bar was calculated from three independent experiments.

the DNAzyme-catalyzed oxidation reaction time of 7 min is selected because a plateau effect is nearly reached after this time. Eventually, the total assay time is much shorter than those of most existing reports (Bi et al., 2013; Li et al., 2010, 2012a; Wu et al., 2013; Yin et al., 2013; Zeng et al., 2013, 2013), including the previously reported DNAzyme-based colorimetric methods (Li et al., 2010).

3.4. Activity analysis of Dam MTase with the proposed colorimetric biosensor

To confirm the ability of the colorimetric biosensor for quantitative detection, various concentrations of Dam MTase were analyzed under the optimized conditions. As can be seen, the color in the reaction solution changed from colorless to green with the decreasing Dam MTase concentrations (left to right, Fig. 3A). Their corresponding UV–vis absorption spectra were shown in Fig. 3B, and the absorption intensity at peak (418 nm) was employed to evaluate signal response of Dam MTase. As the concentration of Dam MTase increased from 0 U/mL to 80 U/mL, the absorption intensity decreased gradually. They were in accord with the fact that more hairpin probes are methylated by Dam MTase, resulting in less production of active G-riched DNAzyme. Fig. 3C depicted the absorption intensity in respond to different concentrations of Dam MTase. As can be seen, a good linear relationship was presented between the absorption intensity of 418 nm and the concentrations of Dam MTase in the range of

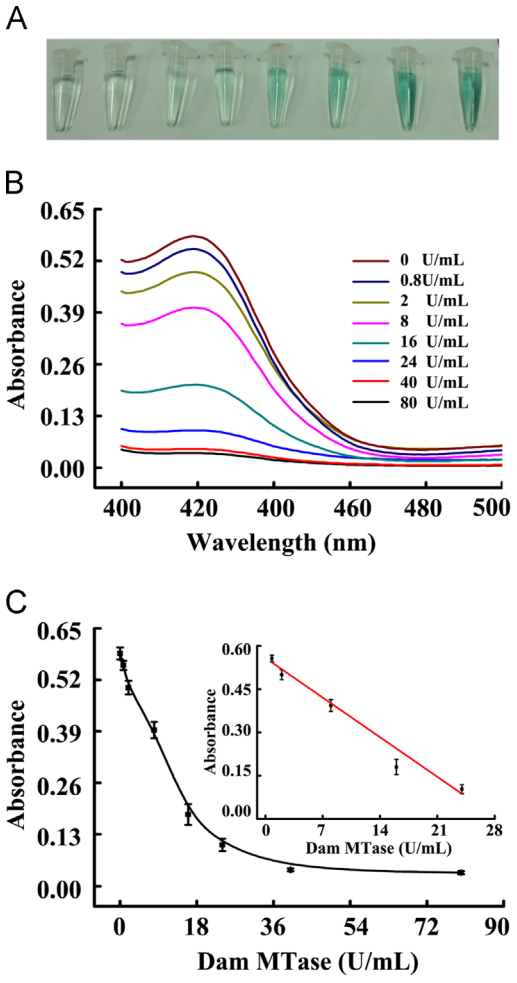


Fig. 3. (A) Photograph and (B) absorption spectra of the system upon incubation with different concentrations of Dam MTase (right to left in A: 0, 0.8, 2, 8, 16, 24, 40 and 80 U/mL, respectively). (C) The corresponding absorption peak intensities versus Dam MTase concentrations. The inset shows the linearity of the absorption response for the sensing system against the low target concentrations.

0.8–24 U/mL (Fig. 3C, inset). With a correlation coefficient of 0.9807, the corresponding regression equation is $A = -0.020\,C + 0.558$, where A and C represent the absorption intensity of 418 nm and the Dam MTase concentration, respectively. A low detection limit of 0.4 U/mL was estimated based on three times the standard deviation of the blank sample signal, which is comparable to that of previous DNAzyme-based colorimetric method (0.25 U/mL) (Li et al., 2010). It is worth pointing out that our sensing strategy was finished within a total assay time of 77 min, at least 2-fold shorter than that of the compared biosensor (about 160 min). Furthermore, this sensitivity is even higher than those of some fluorescence (Li et al., 2007) and chemiluminescence (Bi et al., 2013) approaches.

In addition to the high sensitivity, the proposed colorimetric method is also of high specificity. M.SssI MTase, an enzyme catalyze the methylation of cytosine residues within the double-stranded 5′…CG…3′ sequence, was employ as a control enzyme to demonstrate this property. The tests were performed under the conditions optimized as above, and the comparable results were depicted in Fig. S5. It can be seen that M.SssI MTase caused no more remarkable reduction of absorption intensity than that of the blank sample, and its absorption intensity was much higher than that of Dam MTase. It clearly confirmed that the colorimetric signal was specifically triggered by Dam MTase, which is in agreement with our previous report (Zhao et al., 2013). Therefore, with the use of DNAzyme-based colorimetric assay, the developed biosensor can create a simple and convenient platform for visualized quantitative detection of Dam MTase activity with desirable sensitivity and high selectivity.

3.5. Dam MTase sensing in human serum

The applicability in complex biological matrixes is another significant and challenging factor to estimate the performance of a sensing system. To test the practicality of the proposed approach, diluted human serum samples spiked with Dam MTase were investigated. In this study, the human serum samples were diluted with 1 × NEBuffer 2 in a ratio of 1:10. As depicted in Fig. 4, it was observed that the absorbance values from the 1:10 diluted human serum sample were well consistent with those from NEBuffer 2 sample. The recoveries were in the range of 95.4–104.6% for triple independent measurements with a maximum RSD of 6.5%. Therefore, these results held great potential for the Dam MTase analysis in complex biological samples.

3.6. Dam MTase activity inhibition assay

Since MTases are closely related to cancer and bacterial virulence, inhibitors of MTases activity hold promise as antiproliferative and antibiotic agents. Here, several antibiotics and anticancer drugs including gentamycin, 5-fluorouracil and benzyl penicillin were introduced to estimate the inhibition of Dam MTase activity (20 U/mL). It is worthy to note that other two enzymes (Nt.AlwI and Klenow Fragment) are involved in the sensing system, and the effect of these drugs on them was investigated first. As shown in Fig. S6, all of these drugs caused no distinct change in the absorption intensity compared with the blank. This demonstrates that the proposed system is suitable for the evaluation of Dam MTase inhibition. Then the inhibition tests on Dam MTase were carried out, and the result was presented in Fig. 5. It was observed that the absorption intensity of the sensing system with all drugs increased obviously. This indicated that Dam MTase activity was inhibited by these drugs, which weakened the methylation-blocked effect and led to high colorimetric signal. Furthermore, the dose-dependent inhibition of benzyl penicillin on Dam MTase activity was studied. As the inhibitor concentration increased, the absorption intensity enhanced gradually, implying increasing inhibitory effect on Dam MTase. Therefore, this label-free strategy will be useful for screening inhibitor candidates and MTases-targeted drug discovery.

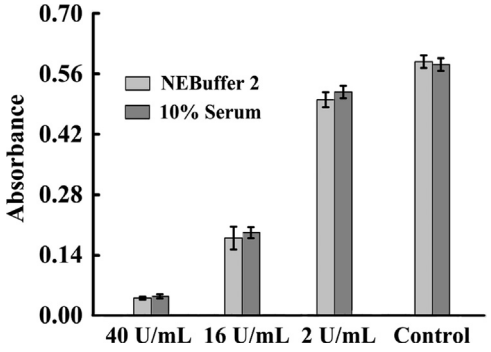


Fig. 4. Results obtained from the testing of NEBuffer 2 and human serum samples without Dam MTase (control) and with Dam MTase, respectively. Human serum was diluted in 1:10 ratio with NEBuffer 2.

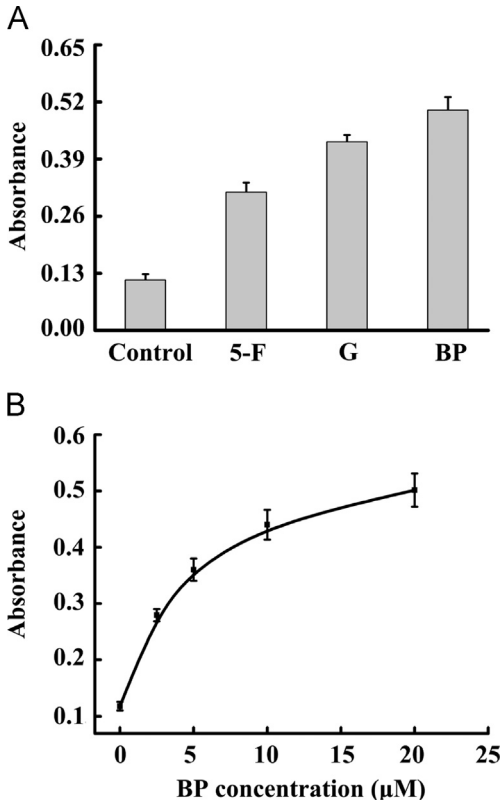


Fig. 5. (A) Influence of 5-fluorouracil (5-F, 250 μM), gentamycin (G, 25 μM) and benzyl penicillin (BP, 25 μM) on the activity of Dam MTase. (B) Inhibition of different concentrations of benzyl penicillin on Dam MTase activity. The benzyl penicillin concentrations were 0, 2.5, 5, 10, and 20 μM, respectively.

4. Conclusions

In summary, we have developed a label-free colorimetric assay for Dam MTase activity, using methylation-blocked cascade amplification of DNAzyme signal, which is free from any complicated procedures or expensive equipments. With only one DNA probe and two enzymes involved, this novel strategy is extremely easy in design. In comparison with previously reported colorimetric assay based on DNAzyme, it is much simpler, more efficient and less costly. In addition, a comparable sensitivity can be achieved within a shorter assay time (77 min to 160 min). Even this low detection limit is superior to those of some fluorescence and chemiluminiscence methods. More importantly, this assay is able to be performed in 10% human serum with satisfying recovery and good reproducibility, holding great potential for further application in complex biology samples. The ability to screen and quantify inhibitors of Dam MTase is also demonstrated with excellent results. By making a minor modification of the hairpin DNA probe and changing the corresponding NEase, the proposed strategy can be readily extended to the detection of other DNA MTases such as cancer-related DNMT family. Given the attractive analytical characteristics and distinct merits, the developed method is expected to be useful for further application in point-of-care diagnostics, drug discovery and disease therapeutics.

Acknowledgements

This research was financially supported by the National Basic Research Program 973 of China (No. 2010CB732603 and No. 2011CB707903), the National Natural Science Foundation of China (No. 21005059) and the China Postdoctoral Science Foundation (No. 2013M530220).

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.2013.11.055.

References

Adams, R.L.P., Rinaldi, A., Seivwright, C., 1991. J. Biochem. Biophys. Methods 22, 19–22.
Bergerat, A., Guschlbauer, W., Fazakerley, G.V., 1991. Proc. Nat. Acad. Sci. U.S.A. 88, 6394–6397.
Bi, S., Zhao, T., Luo, B., Zhu, J.J., 2013. Chem. Commun. 49, 6906–6908.
Boye, E., Marinus, M.G., Løbner-Olesen, A., 1992. J. Bacteriol. 174, 1682–1685.
Brueckner, B., Lyko, F., 2004. Trends Pharmacol. Sci. 25, 551–554.
Chen, F., Zhao, Y., 2013. Analyst 138, 284–289.
Cheng, X., Roberts, R.J., 2001. Nucleic Acids Res. 29, 3784–3795.
Erova, T.E., Pillai, L., Fadl, A.A., Sha, J., Wang, S., Galindo, C.L., Chopra, A.K., 2006. Infect. Immun. 74, 410–424.
Esteller, M., 2007. Nat. Rev. Genet. 8, 286–298.
Garcia-Del Portillo, F., Pucciarelli, M.G., Casadesus, J., 1999. Proc. Nat. Acad. Sci. U.S. A. 96, 11578–11583.
Guo, L., Xu, Y., Ferhan, A.R., Chen, G., Kim, D.H., 2013. J. Am. Chem. Soc. 135, 12338–12345.
Jiang, C., Yan, C.Y., Huang, C., Jiang, J.H., Yu, R.Q., 2012. Anal. Biochem. 423, 224–229.
Lee, J., Kim, Y.K., Min, D.H., 2011. Anal. Chem. 83, 8906–8912.
Li, J., Yan, H., Wang, K., Tan, W., Zhou, X., 2007. Anal. Chem. 79, 1050–1056.
Li, W., Liu, Z., Lin, H., Nie, Z., Chen, J., Xu, X., Yao, S., 2010. Anal. Chem. 82, 1935–1941.
Li, W., Wu, P., Zhang, H., Cai, C., 2012a. Anal. Chem. 84, 7583–7590.
Li, Y., Huang, C., Zheng, J., Qi, H., 2012b. Biosens. Bioelectr. 38, 407–410.
Li, Y., Luo, X., Yan, Z., Zheng, J., Qi, H., 2013. Chem. Commun. 49, 3869–3871.
Liu, T., Zhao, J., Zhang, D., Li, G., 2009. Anal. Chem. 82, 229–233.
Messer, W., Noyer-Weidner, M., 1988. Cell 54, 735–737.
Reisenauer, A., Kahng, L.S., McCollum, S., Shapiro, L., 1999. J. Bacteriol. 181, 5135–5139.
Robertson, K.D., 2005. Nat. Rev. Genet. 6, 597–610.
Song, G., Chen, C., Ren, J., Qu, X., 2009. ACS Nano. 3, 1183–1189.
Su, J., He, X., Wang, Y., Wang, K., Chen, Z., Yan, G., 2012. Biosens. Bioelectr. 36, 123–128.
Szyf, M., 2003. Drug Resistance Updates 6, 341–353.
Szyf, M., 2005. Biochemistry 70, 533–549.
Tian, T., Xiao, H., Long, Y., Zhang, X., Wang, S., Zhou, X., Liu, S., Zhou, X., 2012. Chem. Commun. 48, 10031–10033.
Toyota, M., Ahuja, N., Ohe-Toyota, M., Herman, J.G., Baylin, S.B., Issa, J.P.J., 1999. Proc. Nat. Acad. Sci. U.S.A. 96, 8681–8686.
Tsankova, N., Renthal, W., Kumar, A., Nestler, E.J., 2007. Nat. Rev. Neurosci. 8, 355–367.
Wang, Y., He, X., Wang, K., Su, J., Chen, Z., Yan, G., Du, Y., 2013. Biosens. Bioelectr. 41, 238–243.
Wu, H., Liu, S., Jiang, J., Shen, G., Yu, R., 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.
Yin, H., Zhou, Y., Xu, Z., Wang, M., Ai, S., 2013. Biosens. Bioelectr. 49, 39–45.
Zeng, Y.p., Hu, J., Long, Y., Zhang, C.Y., 2013. Anal. Chem. 85, 6143–6150.
Zhao, Y., Chen, F., Wu, Y., Dong, Y., Fan, C., 2013. Biosens. Bioelectr. 42, 56–61.
Zheng, X.J., Qiu, J.D., Zhang, L., Wang, Z.X., Liang, R.P., 2013. Chem. Commun. 49, 3546–3548.