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Fluorescence biosensor for DNA methyltransferase activity and related inhibitor detection based on methylation-sensitive cleavage primer triggered hyperbranched rolling circle amplification

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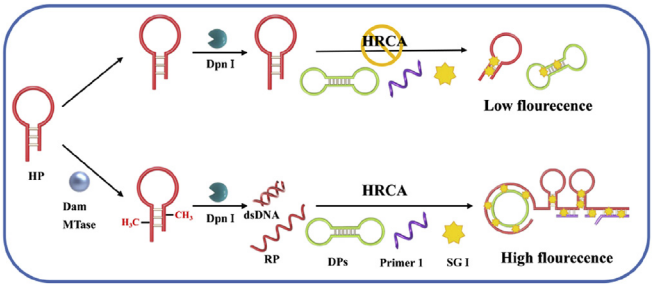
HIGHLIGHTS

- Methylation-sensitive cleavage and hyperbranched rolling circle amplification reaction were implemented for the detection of Dam MTase.
- It can detect Dam MTase at a concentration of 2.5–70 U/mL with a low detection limit of 1.8 U/mL.
- This strategy allows for the selective differentiation of Dam MTase and provides a sensitive platform for screening of Dam MTase inhibitors.

GRAPHICAL ABSTRACT



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ABSTRACT

Highly sensitive and selective detection of DNA adenine methylation methyltransferase (Dam MTase) activity is essential for clinical diagnosis and treatment as Dam MTase can catalyze DNA methylation and has a profound effect on gene regulation. In this study, a fluorescence biosensor has been developed for label-free detection of Dam MTase activity via methylation-sensitive cleavage primers triggered hyperbranched rolling circle amplification (HRCA). A hairpin DNA probe (HP) with a Dam MTase specific recognition sequence on the stem acting as a substrate has been designed. This substrate probe can be methylated by the target in the system and subsequently cleaved by DpnI, which results in the release of the primer release probe (RP) and hence in turn triggers the subsequent HRCA reaction. As the HRCA products contain many double-strand DNA (dsDNA) with different lengths, and the SYBR Green I can be embedded in the dsDNA to produce a strong fluorescence signal. However, in the absence of the target, the presence of the probe HP in the form of a hairpin cannot induce the HRCA reaction, and only weak fluorescence intensity can be detected. Under the optimized conditions, the fluorescence of the system has a linear relationship with the logarithm of the concentration of Dam MTase in the range of 2.5–70 U/mL with a detection limit of 1.8 U/mL. The Dam MTase can be well distinguished from other MTase analogs. The developed sensor was applied to detect target in serum and *E. coli* cell lysate, and the standard recovery rates were in the range of 96%–105%. The results showed that this method has great

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potential for assessing Dam MTase activity in complex biological samples. In addition, the method has been applied to detect the related inhibitors with high efficiency.

1. Introduction

DNA methylation is a biological process by which methyl groups are added to the DNA molecule. Which can change the activity of a DNA segment without changing the sequence. When being located on a gene, methylation typically acts as repress transcription. The specific process of DNA methylation is the demethylation of S-adenosyl-L-methionine (SAM) to adenosylmethionine under the catalysis of DNA methyltransferase (MTase), and the removed methyl group is selectively added to the cytosine or adenine residue (N6) of the DNA [1]. It plays an important role in gene regulation and is essential in many biological processes, including genomic imprinting [2], microRNA gene silencing [3], and X chromosome inactivation [4]. However, in a premalignant or tumor cell, because of aberrations in the DNA MTase - interaction partners, the overexpression of DNA MTase in various tumors can lead to hypermethylation [5]. Excessive DNA methylation silences the transcription of tumor suppressor genes, leading to oncogenic activation [5]. For example, Issa et al. found that an increased DNA methylation capacity accompanied the increase in DNA-MTase transcripts was observed during progressive stages of colon cancer [6]. Samudio-Ruiz et al. implied that epidermal-growth-factor-receptor (EGFR) activation increased DNA MTase activity acutely and resulted in increased global DNA methylation in ovarian cancer [7]. Kobayashi et al. suggested that dysregulation of DNA MTase and possibly DNA MTase-interacting proteins were among the earliest events in prostate cancer [8]. These outcomes suggest that DNA MTase maybe act as a predictive biomarker closely related to hereditary diseases and cancer. Inhibition of DNA MTase activity to block DNA methylation can provide a useful method for disease treating [9]. Therefore, the development of highly selective and sensitive DNA MTase activity detection methods and the screening of inhibitors are of great significance for clinical diagnosis and treatment.

A variety of methods have been developed for DNA MTase activity measurement, such as PCR-based technologies [10,11], bisulfite sequencing [12,13], raman detection [14,15], colorimetry [16–18], and electrochemical detection [19–21]. Fluorescence detection method has high application value in DNA MTase activity detection due to its advantages of simple operation, low cost, easy to use, high sensitivity and fast response [22]. For example, fluorescence monitoring methods that incorporate molecular beacon-based secondary isotherm exponential amplification and hybridization of strand reactions associated with DNAzymes recycling have been developed for sensitive and rapid determination of DNA MTase activity [23–25]. However, much effort is still needed to develop sensitive fluorescence sensor for DNA MTase activity detection.

To improve the detection sensitivity, it is necessary to find some signal amplification techniques combined with detection methods. Hyperbranched rolling circle amplification (HRCA) technique is an isothermal exponential amplification technique which is achieved by a stepwise cascade of primer extension and strand displacement [26]. The HRCA amplification procedure can be performed under isothermal condition and bring about 10^9-fold signal amplification. This quality ensures high sensitivity and when coupled with other detection techniques such as colorimetry and electrochemiluminescence, it can be applied for the detection of various biomarkers [27–29]. A large number of double-stranded DNA (dsDNA) with different lengths can be generated during HRCA reaction, and the abundance of double-stranded chains can be characterized by the fluorescent indicator SYBR Green I (SG I). This character has been applied to develop a number of fluorescent sensors for different targets, such as aflatoxin A [30], T4 polynucleotide kinase [31], microRNA [32], T-2 toxin [33], and so on, all these sensors show high sensitivity.

In this study, a novel highly sensitive and selective fluorescence biosensor has been developed for DNA MTase activity detection via methylation-sensitive cleavage primer triggered dumbbell probe-mediated HRCA. Typically, a hairpin DNA probe (HP) as a substrate with a sequence specifically recognized by Dam MTase on its stem has been designed. When the target is present, the substrate probe is methylated and subsequently cleaved by DpnI (which is a restriction endonuclease and can specifically recognize the methylated sequence), producing two pieces of DNA, one is a short dsDNA and the other is a long ssDNA (RP) and they can trigger subsequent HRCA reaction. The product of HRCA contains many dsDNA with different length, SG I can be embedded into the dsDNA and hence produces strong fluorescence signal, which can be used to represent the concentration of the target. Due to the specific recognition of HP substrate sites by Dam MTase, this sensing strategy is highly selective. Furthermore, the proposed system has the potential to screen methylation inhibitors and has great potential for assessing Dam MTase activity in complex biological samples.

2. Experimental section

2.1. Materials and reagents

The oligonucleotides used in this work were custom synthesized and purified by Sangon Biotechnology. Co., Ltd. (Shanghai, China). The specific information is shown:

Dumbbell-shaped probes (DPs):

5′-P-ACCTCATTGTA-TAGCCCCCCCCTGAGGTAGTAGGTTGCCTCAACTATACAACCTACT-3'.

Hairpin probe (HP):

5′-AGAGTAT**GATC**AATGAGGTAGTAGGTTGTATAGTT**GATC**A-TACTCT-3'.

Mimetic release probe (RP): 5′-TCAATGAGGTAGTAGGTTGTA-TAGTTGA-3′ Primer 1 (P1):5′-TTGTATAGCCCCCCCC-3'.

*The 5′- end of the DPs is phosphorylated. The parts of the oligonucleotide strands that are complementary are underlined. The Dam MTase recognition site in the hairpin substrate is in bold.

DpnI endonuclease, DNA adenine methylation methyltransferase (Dam MTase), CpG methyltransferase (M.SssI), S-adenosyl-L-methionine (SAM), Bst DNA polymerase large fragment, including their corresponding buffers were obtained from New England Biolabs (Beijing, China). Exonuclease I (Exo I), Exonuclease III (Exo III), including their corresponding buffers were purchased from Thermo (Shanghai, China). Nicotinamide adenine dinucleotide (NAD^+) and *E. coli* DNA ligase accompanied by 10 × *E. coli* DNA ligase buffer and 10 × BSA (0.05%) were provided from Takara Biotechnology Co., Ltd. (Dalian, China). SG I was purchased from

Xiamen Biovision Biotechnology Co., Ltd. (Xiamen, China). dNTP mixture solution was provided by Sangon Biotechnology Co., Ltd. (Shanghai, China). Tris (Hydroxymethyl) aminomethane, sodium chloride (NaCl), and magnesium chloride ($MgCl_2$) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Closed normal human serum was purchased from Solarbio Technology Co., Ltd. (Beijing, China). All other chemicals used in this work are analytical reagent grades, and double distilled water (Milli-Q, Millipore, resistance 18.2 MΩ cm) was used throughout the experimental process. DNA powders were formulated into solution by 1 × B2 buffer (10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, pH 7.4).

2.2. Apparatus and method

Fluorescence spectra were measured using a Hitche F-4600 fluorescence spectrometer (Hitachi Ltd., Japan). The excitation and emission slits width of the fluorescence spectrometer were all set to 5.0 nm, and the photomultiplier tube voltage was adjusted to 700 V. The fluorophore of SG I was excited at 488 nm, and the emission spectra from 500 to 600 nm were collected. The performance of the proposed strategy was evaluated by the fluorescence intensity at 534 nm. All experimental data were determined at room temperature unless otherwise stated. Each point was detected for three times and the average value was used for quantitative analysis.

2.3. Preparation of dumbbell-shaped HRCA template

The DPs solution was annealed at 90 °C for 5 min, followed by a slow drop to room temperature, resulting in a dumbbell-shaped probe with a gap. The ligation reaction of the dumbbell template gap performed in a solution, which contained DPs (1.0 μM), *E. coli* DNA ligase (240 U), 10 × BSA (0.05%, 20 μL), NAD^+ (20 mg/mL, 4.0 μL), 1 × *E. coli* DNA ligase bufferand, and reacted at 37 °C for 2 h, *E. coli* DNA ligase catalyzes the phosphodiester bond formation between an adjacent 5′-phosphate and a 3′-hydroxyl of DNA ends under the action of coenzyme factors NAD^+ and Mg^{2+}, connecting the gaps in the dumbbell-shaped template, followed by inactivation at 65 °C for 10 min to terminate the ligation reaction. Afterwards, the unligated probe was sheared by adding Exo I (2.0 μL, 10 U) and Exo III (20 U) for reaction at 37 °C for 2 h and then inactivated at 80 °C for 20 min to terminate the digestion reaction. The DPs template prepared by the above procedure was stored at 4 °C until use.

2.4. Methylation and cleavage of hairpin probes

HP was dissolved in 1 × B2 buffer and annealed at 90 °C for 5 min, then slowly cooled to room temperature, and the obtained HP solution was stored at 4 °C until use. Methylation of the hairpin probe was carried out in a 30 μL of mixed solution with different Dam MTase concentration (37 °C for 2 h), including HP (2.0 μM), SAM (32 μM), 10 × Dam MTase buffer (3.0 μL), 1 × B2 buffer (10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, pH 7.4). Then, DPnI (2 U), 5.0 μL of 10 × CutSmart buffer, 1 × B2 buffer were added, and the cleavage reaction was completed at 37 °C for 2 h. Finally, the mixture was inactivated at 80 °C for 20 min and the methylation and shear reactions were terminated.

2.5. HRCA reaction and fluorescence detection

After the preparation of DPs template and the methylation and cleavage of HP, the HRCA reaction was performed under the action of Bst DNA polymerase. The HRCA reaction was carried out in a 100 μL of mixed solution containing HP (1 μM, 2 μL), DPs (1 μM, 0.5 μL), dNTP (10 μL), Bst DNA polymerase buffer (10 μL), Bst DNA polymerase (8 U/μL,1 μL), $MgSO_4$ (100 mM, 5 μL), 10 × BSA (0.05%, 2 μL), P1 (10 μM, 0.5 μL), and 1 × B2 buffer. The HRCA reaction in the mixed solution was carried out at 63 °C for 1 h, and then the termination reaction was completed by inactivation at 90 °C for 10 min. SG I (20 × , 4 μL) and water (100 μL) were added to HRCA reaction products (100 μL) and incubated for 15 min at room temperature.

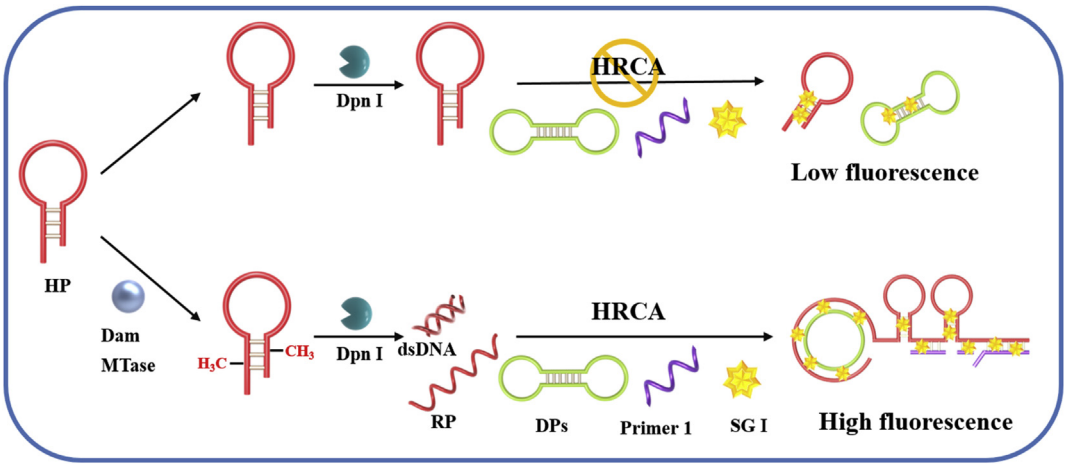
2.6. Culture of bacterial cells

The GW5100 (Dam-positive) and JM110 (Dam-negative) *E.coli* cells were cultured according to previous methods [34]. Briefly, the colonies were inoculated into liquid medium (3 mL, 5 g/L yeast extract, 10 g/L trypsin (10 g/L NaCl)) and shaked at 37 °C in a shaker (250 rpm) and then incubated for 12 h. Then, 30 μL of the cell suspension was added to 3 mL of medium and incubated for 2.5 h. Subsequently, the cell suspension was centrifuged at 5000 rpm to obtain a cell pellet, which was then washed twice with Milli-Q water. The resulting *E. coli* cells were lysed by a RIPA lysis buffer.

3. Results and discussion

3.1. Principle of the fluorescent biosensor for Dam MTase activity detection

Scheme 1 shows the principle of the proposed biosensor for Dam MTase activity detection. This analysis strategy consists of three processes, (1) coupling reaction of Dam MTase and DpnI, (2) performing HRCA amplification reaction, and (3) fluorescence detection. The adenine residue (N6) in the DNA containing the (5′-G-A-T-C-3′) sequence can be methylated by Dam MTase. In the first step reaction, the HP containing a sequence (5′-G-A-T-C-3′) in the stem part which can be specifically recognized by Dam MTase is designed as a substrate. When Dam MTase is added, adenine residue (N6) in the specific sequence is methylated by Dam MTase, the methylated sequence can be specifically recognized and cleaved by DpnI restriction endonuclease. Thus, HP is divided into two parts, one is a short dsDNA and the other is a RP. The HRCA reaction is then initiated in the presence of primer RP, template DPs, Bst DNA polymerase, primer P1, and dNTPs to initiate strand extension and strand displacement, and to produce abundant and diverse lengths of dsDNA and ssDNA. Specifically, the primer RP binds to the template DPs, and the DPs is opened to a circular shape, and the RP is isothermally extended from the 3′ end thereof by the action of Bst DNA polymerase to produce plentiful ssDNA and dsDNA. Primer 1 is a short ssDNA, which is the same as the partial sequence of DPs, so it can complement and pair with the above product, branching the product and hence greatly increasing the content of double-stranded structure. P1 is then extended by Bst DNA polymerase to replace the downstream growing DNA strand, which further provides more binding sites for hybridization of the primer RP. Then, fluorescent signal indicator, SG I, is added to the reaction product, and it can be inserted into the small grooved region of dsDNA and acts as a fluorescent indicator, resulting in strong fluorescent signal. However, DpnI cannot play the role of hydrolyzing in the absence of Dam MTase, RP is not released. And the subsequent HRCA reaction does not occur, resulting in a weak fluorescence signal. The presence of Dam MTase results in the release of RP. This RP triggers HRCA reaction, leading to the enhancement of the fluorescence signal and the intensity is concentration-dependent. On the basis of this principle, the activity of Dam MTase can be monitored by the intensity of fluorescence.



Scheme 1. Principle of HRCA-based fluorescent biosensor for Dam MTase activity detection.

3.2. Feasibility study

Polyacrylamide gel electrophoresis was used to test the feasibility of the proposed method. When Dam MTase was absent, no band of amplification products was observed (Fig. 1A, lane a), the reason lies in that HP is not degraded and thus cannot initiate the HRCA reaction. The short-chain DNA in the reaction system is beyond the visible range of electrophoresis. In contrast, a well-defined band amplification product was observed in the presence of Dam MTase (Fig. 1A, lane b), demonstrating that the hairpin is successfully digested to RP by DpnI and hence triggering HRCA response. To further demonstrate that HP by itself could not cause the amplification, the HRCA reaction was performed directly with HP in the absence of Dam MTase and as expected, no amplification band was observed (Fig. 1A, lane c), indicating the HP itself cannot initiate the HRCA. However, when a mimetic RP was added directly to the HRCA reaction solution, a prominent product band was observed (Fig. 1A, lane d), supporting the hypothesis that HRCA amplification is initiated by RP. These results support the designed approach and its feasibility.

Another method for determining feasibility is fluorescence response. When the target was not present, a weak fluorescence signal was detected (Fig. 1B, curve a), because the DP and HP have a short double-stranded structure, SG I is also embedded in it, generating a background fluorescent signal. The addition of Dam MTase resulted in a significant increase in fluorescence intensity (Fig. 1B, curve b), indicating that HP is degraded and hence releasing the RP and facilitating successful execution of HRCA. All these results demonstrate that it is feasible to proposed a strategy for fluorescent detection of Dam MTase activity by methylation-sensitive cleavage primers trigger HRCA reaction.

3.3. Optimization of the reaction conditions

To obtain high HRCA amplification efficiency and the best sensor performance, the time of HRCA reaction and the concentration of P1, DPs, HP were optimized. The net fluorescence enhancement (ΔF) was used as the indicator. ($\Delta F = F - F_0$, F is the fluorescence intensity in the presence of Dam MTase, and F_0 is the fluorescence intensity in the absence of Dam MTase). The HRCA reaction time was optimized first as it is a direct influencing factor of amplification efficiency. As shown in Fig. 2A, the background fluorescence did not change significantly with increased reaction time. In contrast, an appreciable increase of intensity was observed from 10 to 60 min, and plateau was reached at 60 min. So the optimum reaction period was selected as 60 min.

Furthermore, P1 is a reaction material that sufficient P1 can ensure high HRCA efficiency. So the concentration of P1 was also optimized. With the increasing of P1 concentration, the background fluorescence did not change greatly, but the fluorescence

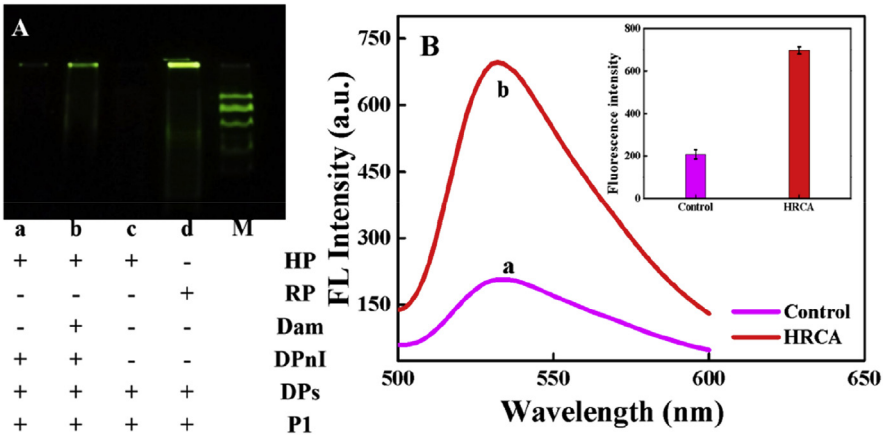


Fig. 1. (A) Non-denaturing polyacrylamide gel electrophoresis: lane M: Marker, lane a: HP + DPnI + DPs + P1, lane b: HP + Dam MTase + DPnI + DPs + P1, lane c: HP + DPs + P1, lane d: RP + DPs + P1. (B) Fluorescence spectra of the analytical method under different conditions: a) HP + DPnI + DPs + P1, b) HP + Dam MTase + DPnI + DPs + P1.

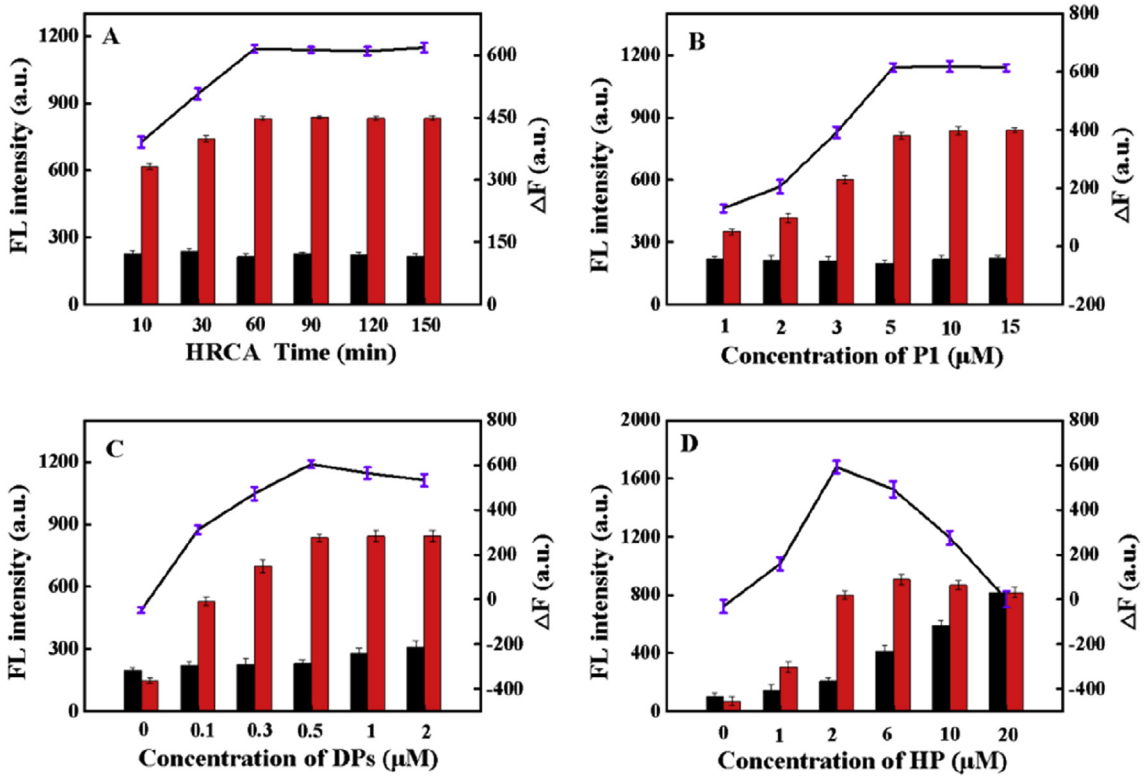


Fig. 2. The effects of (A) HRCA reaction time, (B) P1 concentration, (C) DPs concentration, (D) HP concentration on the fluorescence of the system. Note: The red column represents the amplification signal, and the black column is the background signal. The curves show the net fluorescence intensity enhancement. The concentration of the target used in the optimization experiment is 25 U/mL. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

intensity of the system firstly increased and then stabilized after 5 μM (Fig. 2B). Therefore, the optimum concentration of P1 was chosen to be 5 μM.

The DPs is a key factor which is related to both signal amplification efficiency and background signal. The *F* gradually increased with increasing DPs concentration and reached a maximum intensity at 0.5 μM of DPs. Due to the low concentration of DPs in the reaction mixture and the negative control, the increase in F_0 is not appreciable. So 0.5 μM of DPs was identified as the optimum concentration (Fig. 2C).

HP is used as a substrate to obtain RP after digestion. However, excess amounts of this probe can result in strong background fluorescence if not digested, enabling the optimization of its concentration in the reaction mixture to be essential. As the HP concentration increased, both the background fluorescence intensity and the fluorescence intensity of the system also increased. A maximum fluorescence intensity was observed at 2 μM of HP, while the corresponding background fluorescence was comparable to the background fluorescence when the HP concentrations was unchanged. As more HP was added, the ΔF intensity began to decrease such that ΔF at 0 μM of HP was equal to that of 20 μM of HP. On the basis of these results, 2 μM of HP was used for subsequent experiment.

3.4. Calibration curve for Dam MTase determination

Under the optimal reaction conditions, the fluorescence response of the system was recorded after the addition of different concentration of Dam MTase in 1 × B2 buffer reaction solution (Fig. 3A). The intensity of fluorescence at 534 nm gradually increased with the concentration of Dam MTase (Fig. 3B). This is because more HP probes are methylated and then digested by DPnI upon the addition of Dam MTase, leading to the release of more RP. Furthermore, the fluorescence intensity of the system has a linear relationship with logarithm of the concentration of Dam MTase in the range of 2.5–70 U/mL. The fitted equation is:

$$\Delta F = 514.44\lg C - 115.27 \ (R^2 = 0.992)$$

where *C* is the concentration of Dam MTase (U/mL), and *R* represents the regression coefficient. Herein, the limit of detection (LOD) was 1.8 U/mL according to the equation of $3\sigma_b/s$, where σ_b is the standard deviation of the blank sample, and *s* is the slope of the linear equation. Table 1 suggests that the sensitivity of this biosensor is superior to that of early detection methods for Dam MTase activity, such as colorimetric assay [34,35], light scattering assay [36], this outcome is probably due to the high amplification efficiency of HRCA; And the detection range is comparable with that of some electrochemical [37] or other detection methods [38,39].

3.5. Selectivity of the biosensor

The selectivity of the proposed biosensor was studied (Fig. 4A). Bovine serum albumin (BSA) and M.SssI (which specifically recognizes the 5′-C-G-3′ ssDNA sequence and methylates all of the cytosine residues (C5) therein) were selected as the model interference materials to evaluate the selectivity of the proposed method. When M.SssI was added, the fluorescence intensity did not increase, indicating that the probe could not recognize methylation by M.SssI. And the strategy also showed excellent anti-interference ability against the unrelated BSA. But at present of Dam MTase, the fluorescence intensity of the sensor increased greatly. If a mixture of Dam MTase and interferents had been added, the response has

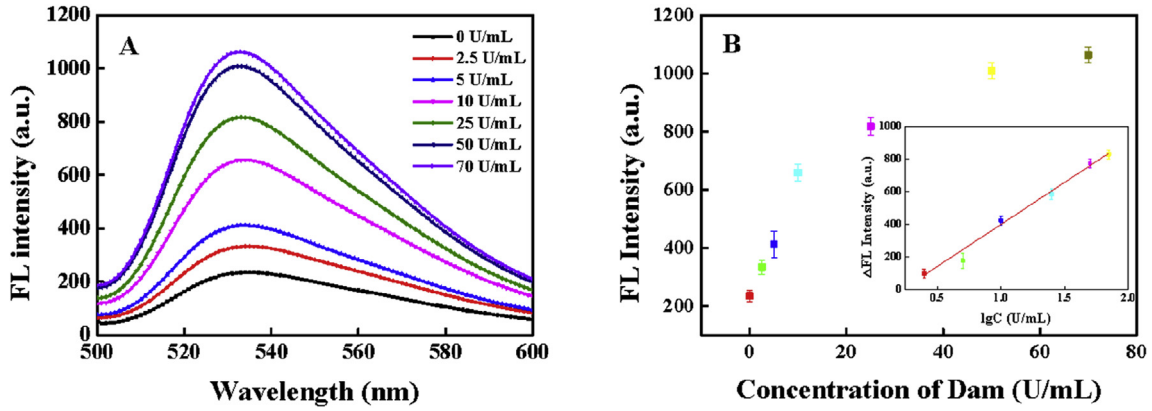


Fig. 3. (A) Fluorescence spectra corresponding to different target concentrations, with Dam MTase concentrations ranging from 0 U/mL to 70 U/mL. (B) Relationship between Dam MTase concentration and net fluorescence intensity *ΔF*. Inset: Calibration curve for the logarithm of *ΔF* and Dam MTase concentrations. Error bars represent the standard deviation of the three replicates.

Table 1
Comparison of the developed method with other methods for the detection of DNA MTase.

Number	Method	Liner range (U/mL)	LOD (U/mL)	Reference
1	Colorimetry	5–120	5	[35]
2	Colorimetry	6–100	6	[36]
3	Light scattering assay	10–100	10	[37]
4	Electrochemical	0.2–10	0.12	[38]
5	Chemiluminescence	1–10	0.52	[39]
6	Fluorescence	1–100	1	[40]
7	Fluorescence	2.5–70	1.8	this work

no obvious difference with that from the addition of Dam MTase. Therefore, the sensor has good selectivity and provides a reliable and effective method for the detection of Dam MTase activity.

3.6. Determination of Dam MTase in complex samples and potential inhibitor screening

To explore the applicability of the detection method in complex biological samples, different concentration of Dam MTase (2.5 U/mL, 10 U/mL, 50 U/mL) was added to the 10% serum samples. Table 2 shows that the proposed fluorescence detection method had a recovery rate of 97.0%–104.0% in serum, and the relative standard deviation (RSD) was in the range of 4.1%–5.5%.

To determine whether this strategy can be applied in real samples, we investigated the endogenous Dam MTase activity of *E. coli* cells using this method. We evaluated Dam MTase activity in Dam-positive *E. coli* GW5100 and Dam-negative *E. coli* JM110. The measurement procedure for Dam MTase detection in *E. coli* cells lysate was the same as that in buffer. Fig. 4B shows that Dam MTase activity was detected in the positive *E. coli* GW5100 cultured for 2.5 h, but it was not detected in the negative *E. coli* JM110 or lysis buffer. The measured fluorescence value was substituted into the regression equation, and the Dam MTase activity in *E. coli* GW5100 lysate was 5.37 U/mL. According to the serum recovery assay, the recovery rate of *E. coli* cell lysate was recorded. Table 2 shows that the proposed fluorescence detection method achieved a recovery rate of 96%–105% in *E. coli* cells lysate, and the RSD was in the range of 4.5%–6.7%. The results show that the method can be effectively and reliably used for the detection of Dam MTase.

As the inhibition of Dam MTase activity is closely related to cancer treatment and antibiotic development, the effectiveness of assessing inhibition of Dam MTase activity was tested. To test the ability of this protocol to screen inhibitors, the anticancer drug 5-fluorouracil was selected as the model for the assay to assess the effect of the drug on the two enzymes (Dam MTase and DpnI) in this strategy. Due to the specific recognition of methylation sequences by DpnI, the effect of 5-fluorouracil on its activity was assessed by absolute methylation of HP. As shown in Fig. 4C, when the concentration of the anticancer drug model was lower than 10 μM, the activity of DpnI was not affected, but because of the toxicity of the anticancer drug, the activity of DpnI was suppressed when the concentration of the drug was as high as 100 μM.

The effect of 5-fluorouracil on Dam MTase activity was then tested. When the Dam MTase activity was inhibited by the anticancer drug, the methylation reaction was blocked. And the amount of DpnI cleavage of HP was insufficient, so the efficiency of HRCA was lower and the fluorescence signal was decreased. The experimental results show that increasing the concentration of the inhibitor 5-fluorouracil is accompanied by a decrease in fluorescence intensity (Fig. 4D). Dam MTase activity is completely inhibited at inhibitor concentration up to 6 μM. Therefore, the proposed sensor strategy has a good prospect in the screening and application of inhibitors.

4. Conclusion

In summary, we have successfully developed a simple, selective and sensitive HRCA-based fluorescent biosensor for the detection of Dam MTase activity. Due to the specific recognition and cleavage of Dam MTase methylated HP by DpnI, the sensor can achieve good selective detection and quantitation of Dam MTase. The proposed sensor has been applied to detect target in complex samples with satisfied results. Additionally, the method reported in this work is able to illustrate Dam MTase inhibition by 5-fluorouracil, an anticancer drug and a model inhibitor used in this study, which has the potential to provide a wide range of therapeutic applications such as drug discovery and disease diagnosis.

Declaration of competing interest

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.

CRediT authorship contribution statement

Liping Chen: Conceptualization, Methodology, Resources, Investigation, Validation, Data curation, Writing - original draft. **Ying**

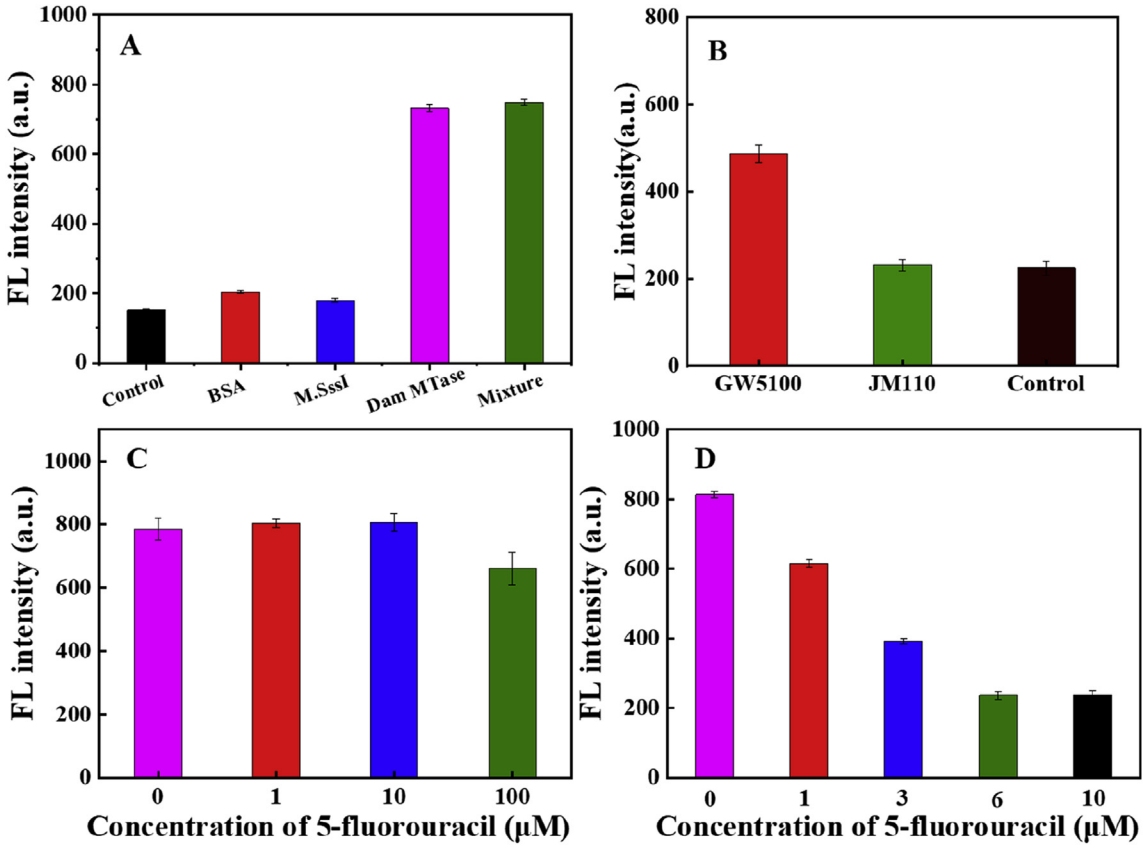


Fig. 4. (A) Fluorescence intensity of this strategy in response to Dam MTase, M.SssI, BSA, Mixture and the control group only used buffer. The concentration of Dam MTase, M.SssI, BSA are 25 U/mL. Error bars represent the standard deviation of the three sets of experiments. (B) Fluorescence intensity of this strategy in response to GW5100, MJ110 and lysis buffer. (C) Inhibition effect of 5-fluorouracil on the activity of DpnI. (D) Inhibition effect of 5-fluorouracil on the activity of Dam MTase. The concentration of Dam MTase is 25 U/mL.

Table 2
Determination of Dam MTase in complex samples(n = 3).

	sample (U/mL)	Added (U/mL)	Found (U/mL)	Recovery (%)	RSD (%, n = 3)
	0	2.5	2.61	104	5.5
serum	0	10	10.22	102	4.1
	0	50	48.56	97	4.9
	0	2.5	2.55	102	6.1
JM110	0	10	9.91	99	5.9
	0	50	50.24	105	6.6
	5.37	5	10.11	97	5.3
GW5100	5.37	20	25.71	101	4.5
	5.37	40	43.43	96	6.7

Zhang: Project administration, Writing - review & editing. **Qian Xia:** Project administration, Writing - review & editing. **Fang Luo:** Resources, Project administration, Supervision. **Longhua Guo:** Resources, Project administration, Supervision. **Bin Qiu:** Resources, Project administration, Supervision. **Zhenyu Lin:** Resources, Visualization, Supervision, Project administration, Writing - review & editing.

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