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A target-triggered biosensing platform for detection of HBV DNA based on DNA walker and CHA

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ABSTRACT

Hepatitis B virus (HBV), one of the causative agents of viral hepatitis, may lead to chronic hepatitis, cirrhosis, and liver cancer. In this work, we designed a sensitive and modular biosensing platform for detecting HBV DNA based on a DNA walker that hangs on to surfaces and a catalyst-triggered catalyzed hairpin assembly (CHA). In the presence of HBV DNA, strand displacement reaction between target and double-stranded complex caused the release of walker strand to trigger the DNA walker. Then, a catalyst was free to open the trapped hairpins to form a new double-strand complex, driving the CHA reaction. Thus, a powerful cascade amplification reaction realized in DNA walker and CHA based on toehold-mediated strand displacement reaction in this system. To achieve quantitative detection of HBV DNA, a fluorescent-quencher signaling pair was employed, the turn-on fluorescence provided an analytical signal. A wide detection range from 0.5 nM to 50 nM with a detection limit as low as 0.20 nM was reached on the condition of acceptable specificity and reproducibility. We could also further apply it to multiple different bioanalysis by changing adjustable elements. This reported biosensor opened a new avenue for sensitivity and modularity of DNA detection.

1. Introduction

Hepatitis B virus (HBV) is a blood-borne pathogen responsible for chronic hepatitis, cirrhosis, and liver cancer [1]. Currently, taking anti-HBV drugs is the most important and effective choice to inhibit viral replication. Anti-HBV drugs like lamivudine, have been widely used for the treatment of chronic hepatitis B (CHB) [2]. However, continuous drug therapy may become a thorny problem in clinical therapy due to gene mutation and selection of resistant strains. This requires that other measures should be taken to deal with HBV. The DNA of HBV, an important biomarker for HBV infection, can vary from a few to more than 109 copies/mL in serum, and it is usually detected in serum [3]. When infecting HBV, the concentration of HBV DNA increases in serum, providing a basis for early diagnosis of HBV. Therefore, DNA detection may become an important method for the early diagnosis of HBV.

The development of DNA detection technology has been the subject of intensive attentions for wide applications [4] owing to its important roles in pathogen analysis, genetic disorder diagnosis, and forensic tests [5]. More recently, several types of strategies have been developed to detect the HBV DNA, such as nanomaterial-based amplification [6–8], electrochemistry [3,9,10], fluorescence [11–14]. Despite the advantages and success in the detection of HBV DNA, these strategies suffer from either complex operation (synthesis of nanomaterial, preparation of biosensor) or lack of effective amplification system, which limit their further use in bioanalysis. Compared to above detection technologies, particularly in terms of fields of application, our system might be well suited to POC (point of care) use, which might be further enhanced by the use of magnetic particles, enabling omission of centrifugation steps and facilitating automation.

Signal amplification is a key component of molecular detection [15]. Molecular machines have previously been designed that are propelled by DNAzymes, protein enzymes and strand displacement [16], which can be adapted to signal-amplification. We have designed a simplified walker powered by Nb. BbvCI on the surface of microparticles, and apply it to the detection of HBV DNA. The walker consists of Nb. BbvCI, a walker strand (W) and a DNA coated on microparticles

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(1 μm) surface that hybridizes with complementary W. W walkers upon the addition of Nb. BbvCI, which selectively hydrolyzes hybridized DNA but not single-stranded DNA (ssDNA). And the driving force for movement is derived from the free energy of binding new ssDNA [17]. In this walker, the specific signal transduction product is generated by the hydrolysis of the substrate by Nb. BbvCI. Thus, the walker will not be plagued by the movement of W that occurs during continuous amplification reaction and a corresponding loss in specificity due to the specificity of Nb. BbvCI. Hence, this walker has almost no meaningful background signal while continuing to move on the microparticles surface.

To obtain a strong signal in a short time, a nucleic acid circuit—the catalytic hairpin assembly (CHA) was employed in this system for further signal-amplification. This CHA circuit originally developed by Yin et al. has proven to be extraordinarily versatile [16,18]. In CHA, two hairpins are kinetically trapped, but in the presence of a single-stranded catalyst from enzyme-catalyzed product of walker, the single-stranded catalyst can undergo strand displacement reaction, leading to the formation of a double-stranded nucleic acid and recycling of the catalyst [16]. The CHA is powerful enough to achieve the signal amplification based on only strand displacement reaction. With the addition of signaling pair, the turn-on fluorescence provides an analytical signal for the detection of target.

In this work, two practical analytical features in this system should be considered. One is that the reaction must be efficient enough to achieve a high level of signal in a relatively short time. The other has to do with the possibility to optimize the design of the sensors to minimize background fluorescence and increase sensitivity.

Inspired by previous reports, we proposed a sensitive and modular biosensor for the detection of HBV DNA. Based on the negligible background and powerful amplification of DNA walker and CHA, the biosensor successfully achieved the quantitative detection of HBV DNA with low background and high sensitivity. Most importantly, this strategy could be modular to the application of detecting other targets by altering the corresponding DNA or aptamer sequence.

2. Materials and methods

2.1. Reagents and materials

All chemicals were purchased from Sigma-Aldrich Chemical Co. unless otherwise indicated. All oligonucleotides were synthesized by Sangon and the sequences were summarized in Table 1. Nb. BbvCI (5′-GC$^{\downarrow}$TGAGG-3′) and 10 × Smart® Buffer were purchased from New England Biolabs Inc. Microparticles (10 mg/mL, 1 μm) were purchased from Bangs Laboratories. Binding buffer (20 mM Tris, 1 M NaCl, 1 mM EDTA, 0.0005% TritonX-100, pH 7.4) was used as hybridization buffer. CST buffer (1 × CutSmart® Buffer, 0.01% TritonX-100, pH 7.9) was used as walker work buffer. All experiments were performed at room temperature (25 ± 2 °C) unless otherwise indicated.

2.2. Instrumentations

A Cary Eclipse Fluorescence spectrophotometer (Agilent, USA) was used to record fluorescence signals. An electrophoresis apparatus (DYY-6C, LIUYI, China) was used in electrophoresis experiment. An imaging system (Bio-Rad Laboratories, USA) was used to analyze gel image. The scanning electron microscope (SEM) (S-3000 N, Hitachi, Japan) was used to analyze the microparticles.

2.3. Preparation of W-Cp1-MP, Cp2-MP, H1 and H2

For the preparation of W-Cp1-MP, a portion of 5 μl of 20 μM Cp1 and W were incubated at 37 °C for 30 min with the same condition of volume and concentration. Then, streptavidin-coated microparticles (50 μl) from stock solution were washed once with 50 μl binding buffer by centrifuging the microparticles at 10,000 rpm for 3 min, followed by resuspension in 50 μl binding buffer. After that, a 10 μl volume of the W-Cp1 complex in binding buffer was added into the microparticles. After incubating for 15 min at 37 °C on a rotator, the sample was washed three times with 50 μl binding buffer to remove unbound complex by centrifuging the microparticles at 10,000 rpm for 3 min, followed by resuspension in 50 μl CST buffer. And the preparation of Cp2-MP was similar to above mentioned. A 10 μl volume of Cp2 in binding buffer was added into the resuspended microparticles. After incubation for 15 min at 37 °C on a rotator, the sample was washed three times with 50 μl binding buffer by centrifuging the microparticles at 10,000 rpm for 3 min, followed by resuspension in 50 μl CST buffer. In the CHA reaction, H1 and H2 in binding buffer were refolded by heating to 95 °C for 5 min, followed by slowly decreasing the temperature to 23 °C at a rate of 0.1 °C s^{-1}.

2.4. Detection of fluorescence

The detection of fluorescence intensity was performed under specific parameter conditions. To record the fluorescence endpoint measurements, the excitation wavelength was fixed at 480 nm and emission spectra were recorded between 500 and 600 nm. In the monitoring of real-time kinetics, the excitation wavelength and the emission wavelength was fixed at 480 nm and 520 nm, respectively. For all fluorescence detections, the width of excitation slit and the emission slit was 5 nm and 10 nm, respectively, all other parameters took the default.

2.5. Analysis of target

For detecting HBV DNA, a portion of 45 μl of HBV DNA in CST buffer was added to 5 μl of W-Cp1-MP in CST buffer, and incubating for 30 min at 37 °C on a rotator. Then decanting 44 μl of the supernatant by centrifuging the microparticles at 10,000 rpm for 3 min, followed by adding 5 μl of Cp2-MP into the supernatant. The walker continuously traveled on the microparticles surface at 37 °C when immediately adding 1 μl of Nb. BbvCI into above mixture. After incubating for

Table 1
Oligonucleotides designed in this study.

Oligonucleotides	Sequence (from 5′ to 3′)
HBV DNA (T)	TGGGAGGAGTTGGGGGAGGAGATTAGGTTAAAGGT
walker strand (W)	CCTCAGCAAAACCAAAAAACCAGTTGGGGGAGGAGAT
Capture probe 1 (Cp1)	Biotin - TTTTTTTTTTACCTTTAACCTAATCTCCTCCCCCAACTCCTCCCA
Capture probe 2 (Cp2)	Biotin - TTTTTTTTTTGGTTTTTTGGTTTTGC$^{\downarrow}$TGAGGCGACATCTAACCTAGCTCACTGAC ($^{\downarrow}$represents the identification point)
Hairpin 1 (H1)	GTCAGTGAGCTAGGTTAGATGTCGCCATGTGTAGACGACATCTAACCTAGCCCTTGTCATAGAGCAC
Hairpin 2 (H2)	AGATGTCGTCTACACATGGCGACATCTAACCTAGCCCATGTGTAGA
F	FAM - CGAGTGCTCTATGACAAGGGCTAGGTT
Q	CCCTTGTCATAGAGCACTCG - Dabcyl
1C-base mismatch target (T–1C-M)	TGGCAGGAGTTGGGGGAGGAGATTAGGTTAAAGGT
2C-base mismatch target (T–2C-M)	TGGCAGCAGTTGGGGGAGGAGATTAGGTTAAAGGT
3C-base mismatch target (T–3C-M)	TGGCAGCAGTTGGCGGAGGAGATTAGGTTAAAGGT