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Cascaded multiple amplification strategy for ultrasensitive detection of HIV/HCV virus DNA

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ABSTRACT

Ultrasensitive detection of HIV and HCV virus DNA is of great importance for early accurate diagnostics and therapy of HIV virus-infected patients. Herein, to our best knowledge, it is the first to use DNA cascaded multiple amplification strategy for ultrasensitive detection of HIV virus DNA with G-quadruplex-specific fluorescent or colorimetric probes as signal carriers. The developed strategy also exhibited universal applicability for HCV virus DNA detection. After reaction for about 4 h, high sensitivity and specificity can be achieved at both fluorescent and colorimetric strategies (limit of detection (LOD) of 10 fM and 0.5 pM were reached for fluorescent and colorimetric detection, respectively). And the single-based mismatched DNA even can be distinguished by naked eyes. It is believed that the cascaded multiple amplification strategy presents a huge advance in sensing platform and potential application in future clinical diagnosis.

1. Introduction

Blood tests are conventional diagnostic methods for HIV infection, including enzyme immunoassay (EIA) (Janssen et al., 1998), enzyme-linked immunosorbent assay (ELISA) (Karlsson et al., 1991), and Western blot test. Using the above-mentioned techniques, the tests for HIV infection are accurate but time-consuming due to the need of a long period to generate antibodies (Clerici et al., 1992; Tai et al., 2000). Different from blood tests, genetic tests attract increasing attention since the strategy can check the virus itself in the absence of antibodies of HIV, which makes it possible to detect HIV virus DNA in real-time once the patient is infected (Guo et al., 2013). To improve the survival rate, reduce the mortality, and decrease therapy cost, it has reached on a consensus that developing universal and cost-effective detection approaches for ultrasensitive detection of target virus DNA (Cao et al., 2015; Chen et al., 2015; Service, 1998; Sun et al., 2015; Zhang et al., 2015b).

Over the past decades, polymerase chain reaction (PCR) has evolved as the most frequently used technique for genetic tests due to the high sensitivity and specificity (Saiki et al., 1988). However, it is hard to apply to short-length DNA due to the difficulty in primer design. Various strategies have been developed for the gene test and medical diagnosis, such as electrochemical (Wang et al., 2014a) and optical aptasensors (Bi et al., 2010; Jiao et al., 2012; Liu et al., 2015; Wang et al., 2015) based on different amplification strategy with the utilization of various nucleases (Duan et al., 2013; Zhang et al., 2014). The strategies have been widely employed for the autocatalytic amplified detection toward different biomolecules. However, the labeled signal reporter requires complicated chemical modification, which is also high cost. Recently, G-quadruplex, a kind of nucleic acid structure that could enhance the fluorescence of NMM (N-methylmesoporphyrin IX) (Li et al., 2014; Wang et al., 2014b) or form DNAzymes with hemin (Li et al., 2013; Zhang et al., 2015a; Zhou et al., 2014), has attracted growing interests because of its inherent advantages of low-cost and simple synthesis.

It is reported that about 1/3 of HIV infected patients were infected by HCV (Hernandez and Sherman, 2011) since human immunodeficiency virus (HIV) and hepatitis C virus (HCV) have a common transmission path. There are about 10 million HIV/HCV-coinfected persons worldwide (Thomas, 2008). With the wide application of the combined antiretroviral therapy, the mortality rate of Acquired Immunodeficiency Syndrome (AIDS) has decreased significantly (Hernandez and Sherman, 2011). However, Patients with HCV infection, co-infected with HIV could accelerate

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the course of liver fibrosis (Di Biagio et al., 2012), and liver disease associated with HCV is still one of the major causes of death for HIV infected patients (Naggie and Sulkowski, 2012). To reduce the mortality of the HIV/HCV-co-infected persons, it is urged to improve hepatitis C prevention and treatment in HIV infected persons. Thus, construction of a platform for ultrasensitive detection of HIV/HCV virus DNA at an early age becomes more and more important and needs further efforts. While, to the best of our knowledge, few works reported multiple enzyme-assisted DNA amplification based ultrasensitive detection of HIV and HCV virus DNA with dual-responsive signals. We reported herein a new cascaded multiple amplification strategy (CMAS) for ultrasensitive detection of target HIV/HCV virus DNA. Once the target DNA was added, it could trigger a series of automatic DNA reaction, generating amplified signal response for sensitive genetic detection. Our developed amplification strategy achieved high sensitivity and specificity with both fluorescent and colorimetric methods. And the single-base mismatched DNA even can be distinguished by naked eyes. The label-free and universal amplification strategy avoids the complicated operation, expensive instruments and sophisticated design, having a prospective application on genetic tests and disease diagnosis and therapy.

2. Experimental section

2.1. Materials

DNA oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co (Shanghai, China). DNA oligonucleotide solutions were prepared by dissolving DNAs in ultrapure water and quantified by measuring UV–visible absorption spectroscopy at 260 nm. Table S1 in the Supporting information shows the sequences of the oligonucleotides used in the study. Klenow fragment, dNTP, Exonuclease III (Exo III), and Nt.BbvCI nicking enzyme were purchased from New England Biolabs Inc. TMB (3,3′,5,5′-tetramethylbenzidine) and NMM (N-methylmesoporphyrin IX) were purchased from Sigma-Aldrich (St. Louis, MO). The other chemicals were purchased from Aladin (Shanghai, China) and used as received without further purification. All other chemicals not mentioned here were analytical reagent grade and used as received. Milli-Q ultrapure water (18.2 MΩ) was used through the experiment.

2.2. Apparatus

Absorbance measurements were performed on a Cary 500 Scan UV/Vis/NIR Spectrophotometer (Varian, USA). Fluorescence measurements were carried out on a F-7000 fluorescence spectrophotometer (Hitachi, Japan). The fluorescence spectra were recorded at room temperature by irradiating NMM at 399 nm.

2.3. Cascaded multiple amplification strategy (CMAS)

The working solution included 0.5 μM DNA-1 and DNA-2. The solution was annealed by heating at 90 °C for 10 min and then cooled down slowly to room temperature. Subsequently, the dNTPs (0.5 mM), Nt.BbvCI nicking enzyme (8 U), Klenow fragment (2 U), and different concentrations of target DNA were added in the CutSmart Buffer (20 mM Tris-acetate,50 mM potassium acetate, 10 mM magnesium acetate, 100 μg/ml BSA, pH 7.9). The reactions were performed at 37 °C for 150 min, followed by termination at 80 °C for 20 min, and cooled down slowly to room temperature. The products were then mixed with DNA-3 and EXO III, and incubated at 37 °C for 100 min. Finally, the reactions were terminated at 70 °C for 20 min

2.4. Fluorescent measurement and colorimetric detection assay

The fluorescence spectra were recorded on F-7000 fluorescence spectrophotometer (Hitachi, Japan). The product of CMAS was added with NMM (1 μM) in 25 mM Tris-HCl buffer (50 mM KCl, 200 mM NaCl, pH 7.4), followed by incubation at room temperature for 30 min, then the fluorescent emission spectra from 550 nm to 750 nm were recorded.

For colorimetric assay, hemin solution (500 nM) was mixed with the product of CMAS in MES-Ac buffer (25 mM MES, 50 mM KAc, pH 4.5), then the mixture was incubated at room temperature for 30 min. Finally, H_2O_2 (2 mM) and TMB solution (400 μM) was added to the above solution. After 15 min, the reaction was stopped by adding equal volume of 2 M H_2SO_4. The absorbance was measured using UV–vis spectrophotometer, and the photographs were obtained using Canon 60D camera.

2.5. Human serum samples

The human serum samples were diluted with reaction buffer 100 times prior to detection. Then, the target DNA was detected in these human blood plasma samples following the same procedure.

3. Result and discussion

3.1. Detection principle

As illustrated in Scheme 1, the platform was prepared by the hybridization between DNA-1 with longer sequences and DNA-2 with shorter sequences, forming the duplex of DNA-1/DNA-2. Once the target DNA was added, the single-stranded section of the DNA-1 was complemented by the sequence of the target DNA (T-DNA). Under the function of polymerase and dNTPs, DNA polymerization reaction occurred at the 3'-terminal of T-DNA with the DNA-1 as template, leading to the release of the DNA-2. Meanwhile, nicking endonuclease cutting site of Nt.BbvCI was formed between the target DNA and the new generated DNA-2. Under the action of nicking endonuclease, the recognition part was cut, initiating automatic recycle process of DNA polymerization and then accompanying the displacement of the replicated strand. Thus, with the help of polymerase and nicking endonuclease, more and more DNA-2 strands were released (Recycle I). The released DNA-2 also acted as the trigger of Recycle process II, including reactions with hairpin-structured DNA-3 and EXO III. In the absence of the DNA-2, the DNA-3 was resistant to catalytic digestion by EXO III since its 3'-terminus is single-stranded. While it could hybridize with the DNA-2 to form double-stranded stem. Then, EXO III could play a role to cleave 3'-terminus of the DNA-3, resulting in the release of the DNA-2 and generation of DNA-4. The DNA-4 was designed to contain two regions, the region E of the DNA-4 had the same sequence with that of the DNA-2. Thus, the DNA-4 also functioned as trigger of the digestion of DNA-3 to release more DNA-2 and DNA-4. The region F of the DNA-4 was G-riched and can form G-quadruplex configuration. In brief, the designed automatic cascaded multiple amplification strategy was composed of three cascaded recycles. Firstly, the T-DNA triggers the Recycle I to produce numerous DNA-2. Then, the DNA-2 was utilized as a trigger to initiate the Recycle II, which generated multiplying DNA-4. Finally, the DNA-4 could continue to cause the Recycle III to release more DNA-4. With the designed automatic cascaded multiple amplification strategy, massive DNA-4 with G-quadruplex configuration was generated, which can either significantly enhance the fluorescence signal of NMM or form G-quadruplex/hemin DNAzyme with hemin to catalyze the colorimetric reaction of TMB in the presence of H_2O_2.