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A label-free, G-quadruplex DNAzyme-based fluorescent probe for signal-amplified DNA detection and turn-on assay of endonuclease

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ABSTRACT

A novel G-quadruplex DNAzyme molecular beacon (G-DNAzymeMB) strategy is developed for assays of target DNA and restriction endonuclease. The detection system consists of G-DNAzymeMB strand and a blocker DNA by using the fluorescence of 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) catalyzed by G-DNAzymeMB as a signal reporter. G-DNAzymeMB exhibits peroxidase activity in its free hairpin structure, and forms a catalytically inactive hybrid when hybridized with blocker DNA. Upon displacement of blocker DNA by target DNA or cleavage by restriction endonuclease, G-DNAzymeMB is released and two lateral portions of G-DNAzymeMB form a G-quadruplex structure, resulting in the recovery of catalytic activity which acts as a cofactor to catalyze H_2O_2-mediated oxidation of H_2DCFDA. For DNA detection system, exonuclease III (Exo III)-catalyzed amplification strategy is introduced to improve the sensitivity and target DNA could be detected as low as 0.1 pM. With respect to restriction endonuclease detection system, 0.1 U/mL EcoRI endonuclease could be detected and this method could be easily transported to other restriction endonuclease analysis by simply changing the recognition sequence. These results demonstrate that the proposed G-DNAzymeMB strategy could be used as a label-free, simple, sensitive and cost-effective approach in analysis of target DNA and restriction endonuclease.

1. Introduction

Molecular beacons (MBs) are specially engineered nucleic acid sequences that fold into a hairpin structure. Since introduction in 1996 (Tyagi and Kramer, 1996), they have shown tremendous use in biochemistry, molecular biology and medical sciences (Broude, 2002; Tan et al., 2004). In the traditional format, MBs act like switches that fluorescing upon hybridization with specific nucleic acid targets. While MBs-based detection systems are one of the most successful separation-free probes (Fang et al., 1999; Sokol et al., 1998), there remain some challenging problems. First, MBs need to be labeled with two non-native moieties (i.e., a donor fluorophore and a quencher), thus suffering from problems associated with double labeling such as high cost, low yield, and singly labeled impurities (Yeh et al., 2010). Second, fluorescence enhancement of MBs is generally limited by background fluorescence, which comes from imperfect quenching of donors and conformational fluctuations of the hairpin structure (Bonnet et al., 1998). Consequently, the fabrication of a label-free, sensitive, simple, and low cost MB-based system has become highly focusing.

DNAzymes are a kind of artificial enzymes that exhibit specific catalytic activities. Compared with natural proteinogenic enzymes, DNAzymes are easier to synthesize and modify, higher thermal stability, and less expensive. Besides, the flexibility in mastering the DNAzyme structures by encoding recognition function into DNAzyme sequences makes DNAzymes ideal candidates for developing bioanalytical platforms. Up to now, they have been widely applied in many biochemical reactions such as DNA, RNA, glycosidic bond cleavage (Burmeister et al., 1997; Carmi et al., 1998; Li et al., 2000a,b; Santoro and Joyce, 1997), porphyrin metalation (Li and Sen, 1996), and DNA self-modification (Li and Breaker, 1999; Li et al., 2000a,b; Sheppard et al., 2000). One kind of DNAzymes formed by hemin and a G-quadruplex aptamer (i.e., G-quadruplex-based DNAzyme) can catalyze H_2O_2-mediated oxidation of 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), luminol or H_2DCFDA (Li et al., 2007a,b; Qiu et al., 2011; Travascio et al., 1998, 1999, 2001), accompanied by a color change, chemiluminescent or fluorescent emission. Among them, H_2DCFDA is a commercially available fluorone dye which is non-fluorescent itself and can be oxidized by DNAzymes into fluorescent dichlorofluorescein (with excitation and emission wavelengths centered at $\lambda = 501$ and 520 nm, respectively). It has been reported that H_2DCFDA is a superior alternative to ABTS in bioanalytical detection (Nakayama and Sintim, 2010).

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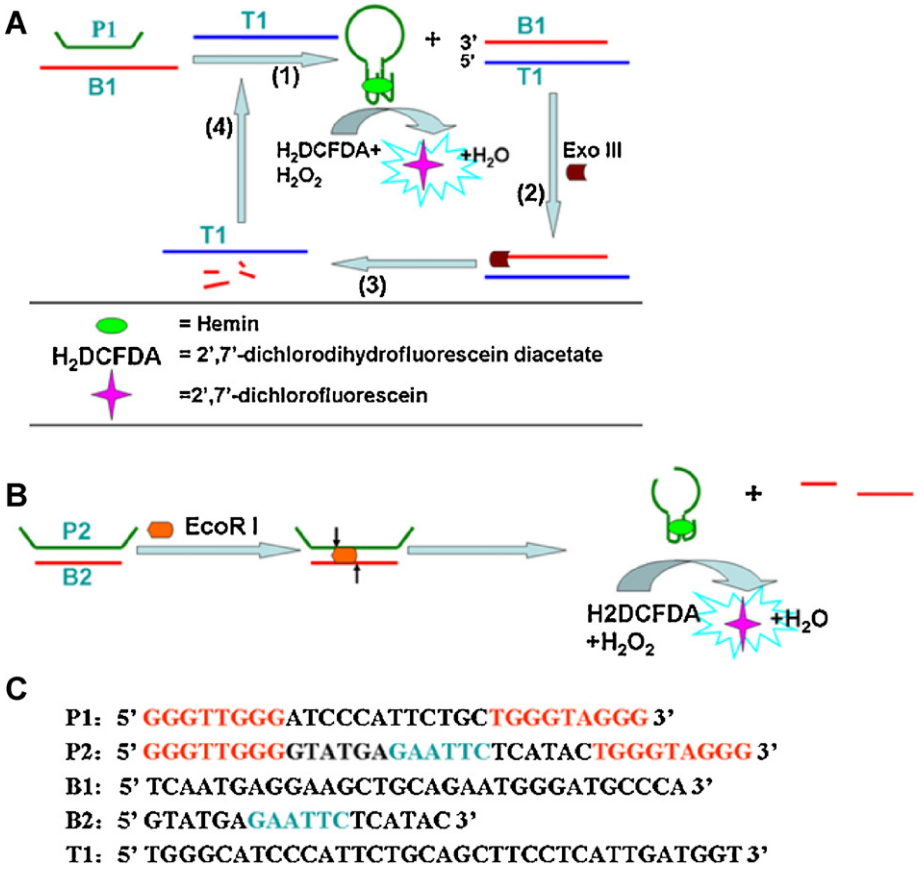

Fig. 1. (A) Schematic illustration of the amplified detection of DNA using label-free G-quadruplex DNAzyme-based molecular beacon. At first, P1 (G-DNAzymeMB1) and B1 (blocker DNA1) formed the catalytically inactive P1/B1 hybrid. T1 (target DNA) hybridized with B1 and released the catalytically active P1 for the generation of fluorescent signals (step (1)); Exonuclease III (Exo III) catalyzed the stepwise removal of mononucleotides from the blunt 3′ terminus of B1 (step (2)), liberating T1 (step (3)); the released T1 triggered amplified cycle by hybridizing with another inactive P1/B1 hybrid (step (4)). (B) Schematic illustration of the assay of the restriction endonuclease. P2 represents G-DNAzymeMB2, and B2 represents blocker DNA2. (C) DNA sequences used in the present work. The G-quadruplex portions were shown in red and the recognition sequences of EcoRI were shown in green.For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

In this study, we construct a label-free, G-quadruplex DNAzyme molecular beacon (G-DNAzymeMB)-based strategy for fluorescent detection of DNA and restriction endonuclease. The fluorescence of H_2DCFDA catalyzed by G-DNAzymeMB is used as a signal reporter. In the "off" state, the central loop portion of G-DNAzymeMB is hybridized with a blocker DNA and formation of G-quadruplex is inhibited. Consequently, catalytic activity of DNAzyme is prevented, resulting in low fluorescent signal. Upon addition of target DNA or enzyme cleavage, two lateral portions of G-DNAzymeMB form a G-quadruplex structure. Then in the presence of hemin, they can catalyze oxidation of H_2DCFDA and give high fluorescent signal.

To improve the sensitivity of DNA detection using G-DNAzymeMB, exonuclease III (Exo III)-catalyzed amplification strategy is introduced. Exonuclease III is sequence-independent and can catalyze the stepwise removal of mononucleotides from 3′-hydroxyl termini of duplex DNA. Its activity on single-stranded DNA is limited and preferred for blunt or recessed 3′ termini (Zuo et al., 2010). Thanks to the amplification of Exo III, 0.1 pM target DNA could be distinctly detected.

2. Materials and methods

2.1. Chemicals and materials

All oligonucleotides were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China) and their sequences were listed in Table S1. The oligonucleotides were determined using the 260 nm UV absorbance and the corresponding extinction coefficient. H_2DCFDA was purchased from Sigma (Missouri, USA). Exo III (15×10^4 U/mL), EcoRI and Hind III (both 15×10^3 U/mL) were purchased from TaKaRa Biotechnology Co. Ltd. (Dalian, China). Hemin was purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). The stock solution of hemin (1 mM) was prepared in dimethyl sulfoxide (DMSO), stored in darkness at −20 °C. Tris(hydroxymethyl)aminomethane (Tris) was bought from Shanghai Chemical Reagent Company (Shanghai, China). Other chemicals were of analytical grade and used without further purification. Solutions were prepared with deionized water processed with a Milli-Q ultra-high purity water system (Millipore, Bedford, MA, USA).

2.2. Assay procedures for DNA and endonuclease detection

In a typical DNA assay, P1 (G-DNAzymeMB1, 0.5 μM) and B1 (blocker DNA1, 0.5 μM) were dissolved in Tris–buffer 1 (25 mM Tris–HCl, 100 mM NaCl, 5 mM $MgCl_2$). The solution was then heated at 90 °C for 10 min and gradually cooled to room temperature to ensure that nucleic acids were hybridized to each other completely. Afterwards, 2 μL T1 (target DNA), 2 μL Exo III (20 units) and 4 μL 10× Exonuclease III buffer (50 mM Tris–HCl, 5 mM $MgCl_2$, 1 mM DTT, pH 8.0) were added in succession. Cleavage reaction of Exo III was conducted at 37 °C for 40 min. Then hemin (0.1 μM) in Tris–buffer 2 (50 mM Tris–HCl, 20 mM KCl, 150 mM NH_4Ac) was