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The three-way junction DNAzyme based probe for label-free colorimetric detection of DNA

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ABSTRACT

A novel three-way junction DNAzyme based probe has been designed for the colorimetric sensing of target DNA. Specifically, a DNAzyme-linked hairpin DNA is used as a signal probe. In the presence of target DNA, the signal probe, assistant probe and target DNA can hybridize with each other, resulting in the formation of a three-way junction DNA. At the same time, the signal probe is opened and the DNAzyme sequence in the signal probe is dehybridized. Subsequently, in the presence of hemin, the DNAzyme sequence forms a G-quadruplex–hemin complex, which catalyzes oxidation of 2, 2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) by H_2O_2 to the colored $ABTS^{\cdot-}$ radical. The significant color changes can be distinguished visually. By the combination of the hairpin probe and the three-way junction DNA probe, the proposed sensor exhibits high recognition property for single-nucleotide polymorphisms (SNPs). This sensor allows the detection of target DNA at a concentration as low as 0.25 nmol L^{-1}. The proposed sensor is easy to fabricate, which avoids the tedious and expensive labeling procedures, and exhibits high selectivity against single-base mismatched DNA.

1. Introduction

DNAzymes (also called deoxyribozyme or catalytic DNAs) are single stranded DNA molecules of a particular sequence, which possess specific catalytic activities to some chemical reactions. DNAzymes have several advantages over traditional protein enzymes, such as high chemical stability, low cost, simple preparation and easy modification (Breaker, 2000). For these unique properties, DNAzymes have been used in numerous biochemical reactions, such as DNA or RNA cleavage (Burmeister et al., 1997; Carmi et al., 1998), porphyrin metalation (Li and Sen, 1996), and DNA self-modification (Li and Breaker, 1999; Sheppard et al., 2000).

Various kinds of artificial DNAzymes have been screened by using in vitro selection methodologies or SELEX (systemic evolution of ligands by exponential enrichment) techniques (Osborne and Andrew, 1997; Tuerk and Gold, 1990). One interesting example of a catalytic DNAzyme that reveals peroxidase-like activity is a complex between hemin and a G-quadruplex (Travascio et al., 1998). G-quadruplexes are unique higher-order structures, in which G-rich nucleic acid sequences form stacked arrays known as the G-quartets (four guanine bases are connected to each other by hydrogen bonded) (Sen and Gilbert, 1988). Hemin is able to specifically bind to G-quadruplex with high affinity. It has been reported that such G-quadruplex–hemin complexes possess peroxidase-like activity 250 times greater than that of hemin alone (Travascio et al., 1999). This neotype G-quadruplex based DNAzymes exhibit high catalytic activity toward the oxidation of 2,2′-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) by H_2O_2, which causes a color change (Travascio et al., 1998). The use of DNAzymes as catalytic labels for biosensor applications has the advantage of avoiding the tedious and expensive labeling procedures for signal readout. Therefore, the G-quadruplex–hemin DNAzymes have been widely used to colorimetric detection of biomolecules and other small molecules, such as DNA molecules (Nakayama and Sintim, 2009), proteins (Li et al., 2007) and metal ions (Li et al., 2008).

Recently, there has been an increasing interest in the detection of single-nucleotide polymorphisms (SNPs). SNPs are single-nucleotide variations that may be substituted, deleted or inserted in a natural DNA sequence. When SNPs occur, both the structure and the function of the encoded protein are changed which often leads to harmful diseases, such as Tay Sachs (Gravel et al., 1995), cystic fibrosis (Cronin et al., 1996), and thalassemia (Muniz et al., 2000). SNPs are the most common variations in human genome, which occur approximately once per 250–1000 bases in a large

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sample of aligned genome sequence (Collins et al., 1998). The high density and mutational stability of SNPs make them particularly useful DNA markers for identification of complex disease genes (Ye et al., 2001). Therefore, the development of a highly selective method for SNPs detection is of great importance for early disease diagnosis and treatment.

In an attempt to improve the selectivity of DNA detection, hairpin probes have been used in conjunction with DNAzymes to construct novel colorimetric biosensors (Li et al., 2011; Xiao et al., 2004). Hairpin probe is single-stranded DNA that possesses a stem-loop structure. Conventional hairpin probe-based colorimetric sensor employs a G-quadruplex DNAzyme-linked DNA sequence, which forms a stem-loop structure in the absence of target DNA, and results in inactivation of the DNAzyme. However, in the presence of target DNA, the stem-loop structure is opened to produce a catalytically active DNAzyme that leads to generation of a colorimetric signal. Hairpin probes can distinguish mismatches over a wider temperature range than that of linear probes (Bonnet et al., 1999), but they exhibit poor distinguishing capability for single-base mismatch (Demidov and Frank, 2004; Kolpashchikov, 2010), which limits their application in SNPs analysis. Thus, a more selective strategy with high discrimination ability for single-base mismatched DNA still needs to be explored.

The general idea of a junction probe is that two DNA probes are hybridized to one DNA target. A detectable signal can be observed only when the two parts of the probes hybridize to the one DNA target (Kolpashchikov, 2010). Since the two parts of the probes form relatively short (7–10 nucleotides) duplexes with target DNA, the junction probe's selectivity is better than that of hairpin probe. Junction probe can be used for highly accurate detection of SNPs at room temperature without the precise temperature control (Kolpashchikov, 2005). Up to date, some new junction probes have been introduced for SNPs analysis, such as binary DNA probe (Kolpashchikov, 2006), three-way junction probe (Kong et al., 2011; Nakayama et al., 2008) and four-way junction probe (Lake et al., 2010).

In this paper, a new three-way junction DNAzyme based probe has been used for the colorimetric detection of target DNA. A G-quadruplex DNAzyme-linked hairpin probe is specially designed as a signal probe, which avoids the tedious and expensive labeling steps for signal readout. The signal probe is opened after hybridization with target DNA and assistant probe, which is accompanied by the formation of a three-way junction DNA. After addition of hemin, the DNAzyme is activated and then catalyzes the oxidation of ABTS by H_2O_2 to form the colored product $ABTS^{\cdot-}$ for ultraviolet–visible (UV–vis) absorbance detection. The proposed DNA sensor is simple, label free, and exhibits high discrimination ability against single-base mismatched DNA.

2. Materials and methods

2.1. Materials

2,2′-Azinobis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) and HPLC-purified oligonucleotides used in this experiment were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China), and the detail base sequences were shown in Table 1.

TritonX-100, Hemin, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), and DMSO were bought from Sigma Aldrich (St. Louis, MO). The stock solution of oligonucleotides (100.00 μmol L^{-1}) were prepared in ultrapure water and stored at -20 °C. The stock solution of hemin (1.00 mmol L^{-1}) was prepared in dimethyl sulfoxide (DMSO) and stored in the dark at −20 °C. Before use, the oligonucleotides and hemin solutions were diluted to required concentrations with ultrapure water and dilution buffer (25.00 mmol L^{-1} Tris–HCl, pH 8.0, 20.00 mmol L^{-1} KCl, 200.00 mmol L^{-1} NaCl, 0.05% TritonX-100 and 1% DMSO), respectively. ABTS and H_2O_2 were freshly prepared before use. The ultrapure water supplied with a Milli-Q system (18.5 MΩ Millipore, USA) was used throughout the experiment. All reagents were used as received without further purification.

Table 1
Sequence of oligonucleotides used in the colorimetric assay

Oligonucleotide	Sequence (from 5′- 3′)
SP1[a]	AAC CCA GTC AGT GTC CTC AGC GTG GGT TGG GCG GGA TGG GT
SP2[b]	CAA CCC AGT CAG TGT CCT CAG CGT GGG TTG GGC GGG ATG GGT
SP3[c]	CCA ACC CAG TCA GTG TCC TCA GCG TGG GTT GGG CGG GAT GGG T
AP[d]	CGC TGA GGA AAT GGA AAA TCT CTA G
Ta[e]	GCT AGA GAT TTT CCA CAC TGA CT
S1[f]	GCT AGA GAT TTT CCA CAC CGA CT
S2[g]	GCT AGA GAT TTT CCA AAC TTA CT

[a,b,c]Thermodynamic parameters of all oligonucleotides were calculated using integrated DNA technologies (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer).

[a,b,c] Hairpin DNAzyme signal probe: the under line sequences are hybridized to form the stem-loop hairpin structure, the italic letters are G-quadruplex DNAzyme sequence.

[d] Assistant probe.

[e] Perfectly complementary target.

[f] Single-base-mismatched target.

[g] Two-bases-mismatched target (the mismatched bases are underlined).

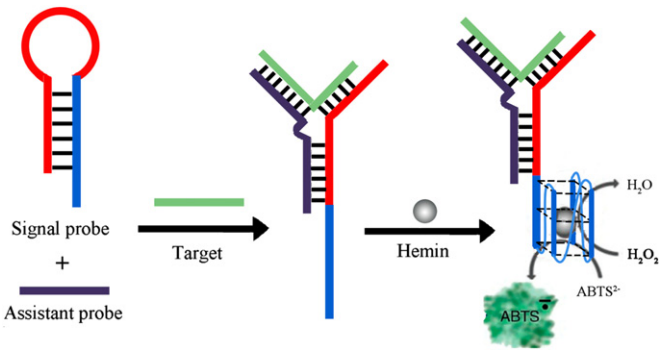

Fig. 1. Colorimetric sensing protocol for target DNA.

2.2. Colorimetric sensing of target DNA

The assay protocol of the colorimetric DNA sensor was depicted in Fig.1. Signal probe was heated to 90 °C for 5 min and then gradually cooled to room temperature to form the hairpin probe before use. First, the assistant probe and target DNA were incubated at 4 °C over night in a hybridization buffer which is containing 20.00 mmol/L Tris–HCl (pH 8.0), 50.00 mmol L^{-1} NaCl and 10.00 mmol L^{-1} $MgCl_2$. Then, signal probe was added and the hybridization was allowed to proceed at 37 °C for 2 h. The above hybridization solution volume was 50 μL. Next, 5 μL hemin (25.00 μmol L^{-1}) and 185 μL HEPES/NH_4OH buffer (25.00 mmol L^{-1}, pH 7.4, 20.00 mmol/L KCl, 100.00 mmol L^{-1} NaCl, and 1% DMSO) were added and incubated at 25 °C for 40 min. It would allow the formation of G-quadruplex–hemin complexes. Finally, 5.0 μL 0.10 mol L^{-1} ABTS and 5.0 μL 0.10 mol L^{-1} H_2O_2 were added to the above reaction mixture to initiate the catalytic reaction. The final concentration of signal probe and assistant probe were 50.00 nmol L^{-1}. The time-dependent