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Label-free and enzyme-free sensitive fluorescent detection of human immunodeficiency virus deoxyribonucleic acid based on hybridization chain reaction

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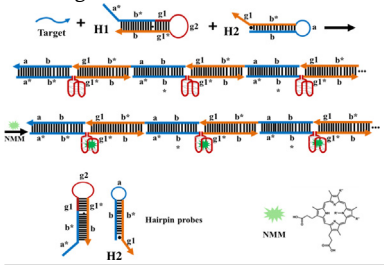
HIGHLIGHTS

- A label-free and enzyme-free sensitive fluorescent detection of HIV DNA was developed.
- G-quadruplexes bound with NMM were used as a versatile signaling reporter.
- DNA nanowires were self-assembled through hybridization chain reaction to amplify the response signal.

GRAPHICAL ABSTRACT

In the presence of target DNA, target DNA is hybridized with hairpin probe H1 from the toe and triggers H1 to be opened. The G-quadruplex sequence in H1 which is originally inactive can form a G-quadruplex structure stabilized by K^+ ions. Then, the remaining sequence in the opened H1 is hybridized with H2 from the toe and triggers H2 to be opened simultaneously. The opened H2 is used as a new initiator to hybridize with a new H1 and triggers the new H1 to be opened. Thus, H1 and H2 are cross-opened successively and self-assembled into a long DNA nanowire incorporated with numerous G-quadruplexes, resulting in the fluorescent enhancement after binding with NMM.



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ABSTRACT

A label-free and enzyme-free sensitive fluorescent detection of human immunodeficiency virus (HIV) deoxyribonucleic acid (DNA) based on isothermal hybridization chain reaction (HCR) was developed. A G-quadruplex sequence which was incorporated into one of the two hairpin probes was inactive in the absence of target DNA. However, at the presence of target DNA numerous G-quadruplexes along DNA nanowires were self-assembled through HCR. Using *N*-methyl mesoporphyrin IX (NMM) as the fluorophore, a "turn-on" fluorescent response would be achieved and detected as low as 0.5 nmol L^{-1} of HIV DNA. This proposed method was applied to detect HIV DNA in biologic samples with satisfactory results.

1. Introduction

The detection of trace amount target DNA is of vital importance in clinical diagnosis, mutational analysis, and gene therapy [1,2]. Therefore, the amplified detection of DNA has attracted substantial research efforts, and a number of enzyme-based amplification strategies such as polymerase chain reaction [3], ligase chain reaction [4], and rolling circle amplification [5] have been extensively employed to improve the sensitivity and lower the detection limit. However, these amplified methods require protein enzymes, which are cost consuming and are sensitive to reaction conditions, such as temperature and pH. Recently, great attention

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has been focused on enzyme-free signal amplification based on hybridization and strand displacement [6]. In particular, isothermal hybridization chain reaction (HCR), which was first introduced by Dirks and Pierce [7], provides a fascinating strategy in DNA sensing applications. In HCR, two species of metastable DNA hairpin probes coexist in solution, which self-assemble into a long nicked double-stranded DNA structure upon the introduction of target DNA. Besides the high amplification, the most distinct advantages of HCR are its controlled kinetics and enzyme-free nature. So far, many sensitive and selective DNA biosensors have been developed by combining the amplified capability of HCR with various signal transduction techniques such as surface plasmon resonance [8] , surface-enhanced Raman spectroscopy [9,10], gold nanoparticles-based colorimetric assay [11], electrochemistry [12,13], electrochemiluminescence [14], quartz crystal microbalance [15], fluorescence [16–20]. Among these methods, fluorescent methods are particularly attractive due to their high sensitivity, easy readout, low sample volume, simple operation and feasible quantification. However, many HCR-amplification fluorescent methods [16–18] are based on the labeling of hairpin probes. Fluorescence labeling brings about complexity and high cost which may limit its practical use. Thus, it is necessary to design a convenient and inexpensive fluorescent signal readout approach with rapid, easy manipulation for the sensitive and selective detection of target DNA.

G-quadruplexes, generally stabilized by alkali metal cations, are unique high-order structures in which G-rich nucleic acid sequences form stacked arrays of G-quartets connected by Hoogsteen-type base pairing [21]. G-quadruplexes can bind with hemin to catalyze the oxidation of peroxidase substrate by H_2O_2 to produce a colored product or bind with porphyrin molecule to enhance its fluorescent intensity as a versatile signaling reporter. Recently, Willner' group [22] and our group [23] utilized HCR to assembly hemin/G-quadruplex horseradish peroxidase (HRP)-mimicking DNAzyme nanowires, which catalyzed the oxidation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) by H_2O_2 to $ABTS^{\bullet-}$, for the amplified colorimetric detection of DNA and heavy metal ion, respectively. Deng and Tang' group utilized ZnPPIX as the fluorophore to develop a G-quadruplex-based HCR for the amplified detection of nucleic acid [24]. Compared with the utility of G-quadruplexes as the DNAzyme, the fluorescent enhancement based on G-quadruplexes has its own unique characteristics, such as higher stability and reproducibility [25] .

N-methyl mesoporphyrin IX (NMM) is a commercially available unsymmetrical anionic porphyrin characterized by a pronounced structural selectivity for G-quadruplexes but not for duplex-, triplex- or single-stranded forms. It is weakly fluorescent, but exhibits a >20-fold enhancement in its fluorescence upon binding to G-quadruplexes. However, similar properties were not evident in the presence of duplex-, triplex- or single-stranded nucleic acid structures [26,27]. Herein, we took advantage of G-quadruplexes as the versatile signaling reporter to develop a new label-free and enzyme-free sensitive fluorescent detection of target DNA based on hybridization chain reaction in which G-quadruplexes were incorporated and bound with the fluorophore NMM. Worldwide-concerned acquired immunodeficiency syndrome (AIDS) is a severe communicable immunodeficiency disease caused by the human immune deficiency virus (HIV) [28]. It is of great value to develop simple, direct label-free nucleic acid-based detection methods for the sequences related to HIV in the early diagnosis of HIV infection. 21-mer single-stranded oligonucleotide from the HIV-1 U5 long terminal repeat (LTR) sequence [28] was detected as target DNA in this study. In the presence of target DNA, two DNA hairpin probes were self-assembled into long DNA nanowires incorporated with numerous G-quadruplexes, resulting in the fluorescent enhancement. The proposed method exhibited high sensitivity and selectivity towards target DNA, which might provide a versatile sensing platform for DNA-based molecular diagnostics.

2. Experimental

2.1. Materials

N-methyl mesoporphyrin IX (NMM) was purchased from J&K Scientific Ltd. (Beijing, China). Tris(hydroxymethyl) aminomethane (Tris) and sodium chloride (NaCl) were bought from Sinopharm Group Co., Ltd. (Shanghai, China). Potassium chloride (KCl) was obtained from Shanghai experiment reagent Co., Ltd. (Shanghai, China). All DNA used in this work (Table 1) were synthesized and HPLC purified by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade.

The DNA solutions were prepared by 20 mmol L^{-1} Tris–HCl buffer (pH 7.2, 400 mmol L^{-1} NaCl). The stock solution of NMM (1.56 mmol L^{-1}) was prepared by dimethyl sulfoxide (DMSO), stored in darkness at −20 °C. Before use, NMM solution was diluted with water to desired concentration.

2.2. Apparatus

The concentration of NMM was measured on a Lambda 750 spectrophotometer (PE, USA) by absorbance at 379 nm assuming an extinction coefficient of 1.45×10^5 mol^{-1} L cm^{-1} [27,28]. Fluorescent measurements with NMM as the fluorophore (excited at 399 nm) were carried out on a Cary Eclipse fluorospectrophotometer (Varian, USA). Gel electrophoresis was performed on a DYY-6C electrophoresis system (Beijing Liuyi instrument factory, China) and photographed in a Bio-Best 200 E gel documentation system (SIM International Group Co., Ltd., China). The water used was purified by a Millipore Milli-Q (18 MΩ cm).

2.3. Procedure for the detection of target DNA

Hairpin probes H1 (5.0 μmol L^{-1}) and H2 (5.0 μmol L^{-1}) were respectively heated to 95 °C for 2 min and cooled down slowly to room temperature for at least 1 h before use. Then 10 μL H1 (5.0 μmol L^{-1}), 10 μL H2 (5.0 μmol L^{-1}), 13 μL KCl solution (1.0 mol L^{-1}) and 10 μL target DNA of different concentrations were added to 142 μL 20 mmol L^{-1} Tris–HCl buffer (pH 7.2,

Table 1
Sequences of DNA used in this work.

Name	Sequence (5′ → 3′)
Target DNA	ACT GCT AGA GAT TTT CCA CAT
Single-base mismatch target (1MT)	ACT ACT AGA GAT TTT CCA CAT
Two-base mismatch target (2MT)	ACT GCT AGA GCT TTT CCA GAT
Three-base mismatch target (3MT)	ACT GAT AGA GCT TTT CCA GAT
Non-complementary target (NCT)	ATG TCG AAA ATC TCT AGC AGT
Hairpin probe 1 (H1)	ATG TGG AAA ATC TCT AGC AGT TGG GTA GGG CGG GTT GGG ATA TAC CCA TCT GCT AGA GAT TTT
Hairpin probe 2 (H2)	ACT GCT AGA GAT TTT CCA CAT AAA ATC TCT AGC AGA TGG GTA

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