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SHORT COMMUNICATION

A Novel Single Fluorophore-Labeled Double-Stranded Oligonucleotide Probe for Fluorescence-Enhanced Nucleic Acid Detection Based on the Inherent Quenching Ability of Deoxyguanosine Bases and Competitive Strand-Displacement Reaction

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Abstract We develop a novel single fluorophore-labeled double-stranded oligonucleotide (OND) probe for rapid, nanostructure-free, fluorescence-enhanced nucleic acid detection for the first time. We further demonstrate such probe is able to well discriminate single-base mutation in nucleic acid. The design takes advantage of an inherent quenching ability of guanine bases. The short strand of the probe is designed with an end-labeled fluorophore that is placed adjacent to two guanines as the quencher located on the long opposite strand, resulting in great quenching of dye fluorescence. In the presence of a target complementary to the long strand of the probe, a competitive strand-displacement reaction occurs and the long strand forms a more stable duplex with the target, resulting in the two strands of the probe being separated from each other. As a consequence of this displacement, the fluorophore and the quencher are no longer in close proximity and dye fluorescence increases, signaling the presence of target.

Keywords Fluorescence · Quenching · Deoxyguanosine · Competitive strand-displacement reaction

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Introduction

It is very important to develop rapid, cost-effective, sensitive and specific methods for the detection of nucleic acid due to their various applications in gene expression profiling, clinical disease diagnostics and treatment [1]. The introduction of simple methods for fluorescent labeling of nucleic acids has opened the door that enables nucleic acid hybridization probes to be used for research and development [2]. Indeed, in recent years, fluorescent probes have multiplied at a high rate and the homogeneous fluorescence assays based on FRET (fluorescence resonance energy transfer) or quenching mechanism for nucleic acid detection have been widely developed [3]. Among such probes, Taqman probes, molecular beacons (MBs), and Scorpions are labeled with both a fluorescent reporter and a quencher dye and the fluorescence is only released from the reporter when the two dyes are physically separated after hybridization occurs. Although extensively used in a broad spectrum of applications [2, 4–7], they still have some drawbacks in that they require labeling at both ends of the OND probe with specific dyes that suffer in overall yield and are not cost-effective [8]. To solve these problems, single fluorophore-labeled probe with only one fluorophore tag has been developed. To signal target detection event, however, structures including gold nanoparticle, single-walled carbon nanotubes, multi-walled carbon nanotubes, carbon nanoparticles, carbon nanospheres, mesoporous carbon microparticles, nano-C_{60}, graphene oxide, poly(*p*-phenylenediamine) nanobelts, polyaniline

nanofibres, poly(*o*-phenylenediamine) colloids, poly(2,3-diaminonaphthalene) microspheres, poly(*m*-phenylenediamine) nanorods, coordination polymer colloids and nanobelts, Ag@poly(*m*-phenylenediamine) core-shell nanoparticles, tetracyanoquinodimethane nanoparticles, and supramolecular microparticles are also used as a "quencher" at the same time [3, 9–33]. It has also been demonstrated that the fluorescence of some fluorescent dyes can be efficiently quenched by an interaction between the dye and a nucleotide base and very effective quenching often occurs between dye and a guanine base via potoinduced electron transfer [34–39].

On the other hand, a dual-labeled double-stranded DNA (dsDNA) probe based on competitive strand-displacement reaction has also been developed and successfully used in homogeneous detection of target without any separation step [40–42]. Such probe consists of two complementary OND strands, one short competitor strand labeled with a nonfluorescent quencher, and the other long strand complementary with target labeled with a fluorophore. When the two strands are hybridized to each other, a contact quenching occurs due to that the fluorophore and quencher are in close proximity. In contrast, the presence of a target, the long strand forms a more stable probe–target hybrid. As a result, the two strands are separated from each other and fluorescence enhancement is observed.

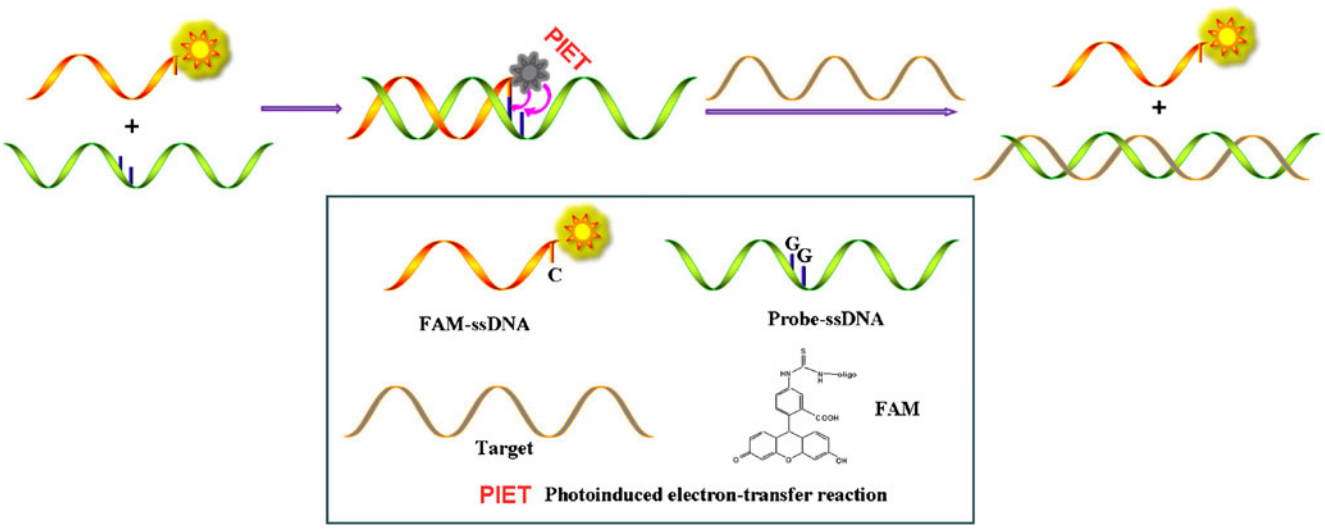
In this communication, we develop a novel single fluorophore-labeled double-stranded OND probe for rapid, nanostructure-free, fluorescence-enhanced nucleic acid detection for the first time. The design takes advantage of an inherent quenching ability of guanine bases and thus the nonfluorescent quencher is eliminated from the probe. The short strand of the probe is designed with an end-labeled fluorophore that is placed adjacent to two guanines located on the long opposite strand, leading to great fluorescence quenching. In the presence of a target which is complementary to the long strand of the probe, a competitive strand-displacement reaction occurs and the two strands of the probe are separated from each other, leading to fluorescence increase. We further demonstrate that such probe is able to well discriminate single-base mutation in nucleic acid.

Experimental Section

All chemically synthesized ONDs were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). DNA concentration was estimated by measuring the absorbance at 260 nm. All the other chemicals were purchased from Aladin Ltd. (Shanghai, China) and used as received without further purification. The water used throughout all experiments was purified through a Millipore system. Fluorescent emission spectra were recorded on a PerkinElmer LS55 Luminescence Spectrometer (PerkinElmer Instruments, U.K.).

OND sequences used are listed below:

(1) The FAM dye-labeled short strand of the probe (FAM-ssDNA):
5′-FAM-CAC ACT GAC T-3′ (FAM = fluorescein-based dye)
(2) The long strand of the probe-ssDNA (P_{HIV}):
5′-AGT CAG TGT GGA AAA TCT CTA GC-3′
(3) The complementary target to P_{HIV} (T_1):
5′-GCT AGA GAT TTT CCA CAC TGA CT-3′



Scheme 1 A schematic diagram (not to scale) to illustrate the nucleic acid detection mechanism using single fluorophore-labeled double-stranded OND as a probe based on the inherent quenching ability of guanine bases and competitive strand-displacement reaction

(4) The single-base mismatched target to P_{HIV} (T_2):
5′-GCT AGA GAT TGT CCA CAC TGA CT-3′
(mismatch underlined).

Results and Discussion

Scheme 1 presents a schematic diagram to illustrate the nucleic acid detection mechanism using single fluorophore-labeled double-stranded OND as a probe based on the inherent quenching ability of guanine bases and competitive strand-displacement reaction. In the absence of target, the fluorescent dye-labeled short strand forms a duplex with the long opposite strand, bringing the dye very close to the two guanines on the long strand. Subsequently, a great fluorescence quenching occurs owing to the photoinduced electron transfer between the dye and the guanines. In contrast, in the presence of a target, a competitive strand-displacement reaction occurs and the long strand forms a more stable duplex with the target. As a result, the two strands of the probe are separated from each other and the dye and the guanines are no longer in close proximity, resulting in a significant fluorescence increase.

To demonstrate a proof of concept that our probe can be used for fluorescence-enhanced nucleic acid detection, we chose an OND sequence associated with human immunodeficiency virus (HIV) as a model system. Figure 1 shows the fluorescence emission spectra of FAM-labeled short strand OND (FAM-OND) of the probe at different conditions. In Tris–HCl buffer, FAM-OND exhibits a strong fluorescence emission

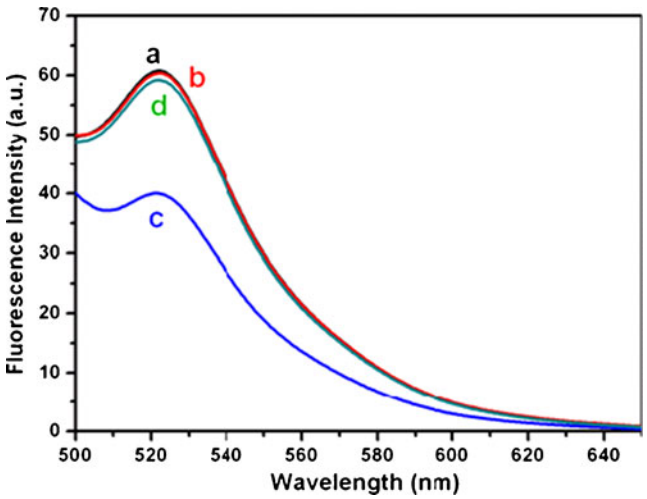


Fig. 1 Fluorescence emission spectra of the FAM-OND (100 nM) at different conditions: **a** FAM-OND; **b** FAM-OND + 300 nM T_1; **c** FAM-OND + 100 nM P_{HIV}; **d** FAM-OND + 100 nM P_{HIV} + 300 nM T_1. Excitation was at 480 nm, and the emission was monitored at 521 nm. All measurements were performed in Tris–HCl buffer (pH: 7.4, $MgCl_2$: 5 mM)

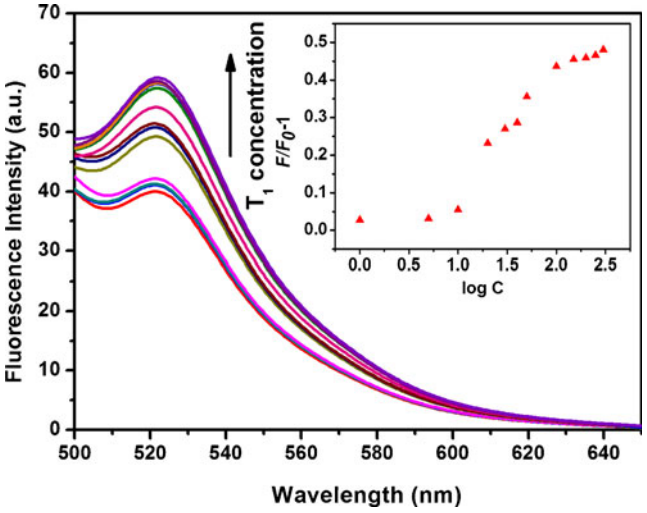


Fig. 2 Fluorescence emission spectra of the probe (100 nM) (FAM-OND + P_{HIV}) in the presence of different concentration of T_1 (from bottom to top: 0, 1, 5, 10, 20, 30, 40, 50, 100, 150, 200, 250, 300 nM). Excitation was at 480 nm, and the emission was monitored at 521 nm. Inset: fluorescence intensity ratio of FAM-OND with F/F_0-1 (where F and F_0 are the fluorescence intensity with and without the presence of T_1, respectively) plotted against the logarithm of the concentration of T_1

at 521 nm which can be attributed to the presence of the fluorescein-based dye (curve a). In the presence of long opposite strand P_{HIV}, about 34% quenching of the fluorescence emission is observed (curve c), indicating that a duplex is formed and the two guanine bases in the middle of P_{HIV} can effectively quench the dye fluorescence dye of FAM-OND due to their very close proximity to each other and the subsequent occurrence of photoinduced electron transfer from dye to the guanine bases. It should be pointed out that our measurement was performed right after the addition of P_{HIV} into the FAM-OND solution and the involvement of

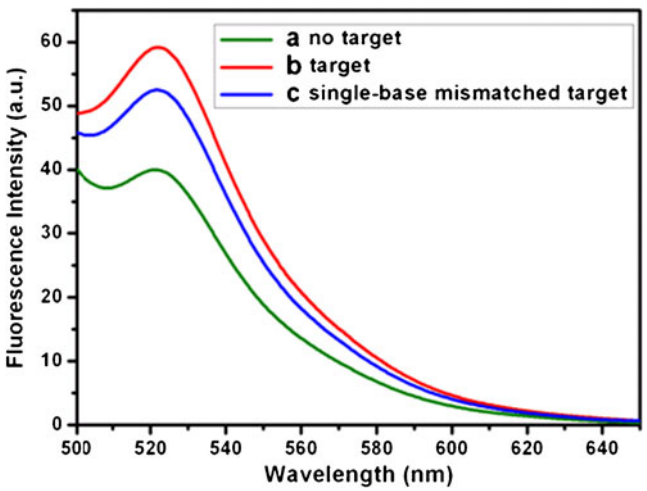


Fig. 3 Fluorescence emission spectra (λ_{ex} = 480 nm) of the probe (100 nM) at different conditions: **a** Probe; **b** Probe + 300 nM T_1; **c** Probe + 300 nM T_2

longer incubation time does not lead to an observable decrease of the fluorescence emission, indicating that the quenching process is very rapid. In contrast, a significant fluorescence enhancement is observed after the introduction of complementary target T_1 into the double-stranded probe solution over a 1-min period, leading to a 97.4% fluorescence recovery (curve d). These observations indicate that the nucleic acid detection is very fast and convenient. It is also worthwhile mentioning that the fluorescence of the free FAM-OND was, however, scarcely influenced by the addition of T_1 in the absence of P_{HIV} (curve b).

We also collected emission spectra of the probe in the presence of different concentrations of T_1 ranging from 1 to 300 nM, as shown in Fig. 2. The inset of Fig. 2 illustrates the fluorescence intensity changes (F/F_0-1) of the probe upon addition of different concentrations of T_1, where F_0 and F are probe fluorescence intensities at 521 nm in the absence and the presence of T_1, respectively. It is clearly seen that the fluorescence intensity of the probe is sensitive to T_1 and increases as the concentration of T_1 increases from 1 to 300 nM. It should be noted that when the concentration of T_1 is higher than 100 nM, more than 90% of the fluorescence recovery can be observed.

The selectivity of the probe was also evaluated by examining its fluorescence responses toward T_1 and the single-base mismatched target T_2. The F/F_0 value obtained upon addition of 300 nM of T_2 is about 88.7% of the value obtained upon addition of 300 nM of T_1 into the probe solution, as shown in Fig. 3. Such observation demonstrates that our present probe is able to well discriminate single-base mutation in nucleic acid.

Conclusions

A novel single fluorophore-labeled double-stranded OND probe for rapid, nanostructure-free, fluorescence-enhanced nucleic acid detection is developed for the first time. The detection is based on the inherent quenching ability of guanine bases and competitive strand-displacement reaction. Our design is simple and cost effective and the detection is convenient and fast. Such probe is very promising for single-base mutation detection in nucleic acid.

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