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A fluorescence polarization assay for nucleic acid based on the amplification of hybridization chain reaction and nanoparticles

Jingjin Zhao[a,*], Zhidan Chu[b], Xue Jin[b], Shulin Zhao[b,*]

[a] Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection of Ministry Education, Guangxi Normal University, Guilin 541004, PR China
[b] Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources of Education Ministry, Guangxi Normal University, Guilin 541004, PR China

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ABSTRACT

An amplified fluorescence polarization (FP) assay based on hybridization chain reaction (HCR) and nanoparticles was developed for the detection of nucleic acid. In this work, the target nucleic acid could trigger the autonomous cross-opening the biotin-modified hairpin probe H1 and fluorescein-labeled hairpin probe H2 to form long fluorescent nicked double-strand polymers (dsDNA). Thus the FP value was increased because the rotation rate of fluorescein slowed down by the formation long HCR products with a larger molecular mass. After then introduction of streptavidin-coated the SiO_2 nanoparticles (SA-SiNPs), the HCR-produced dsDNA products and SA-SiNPs were combined via the interactions of biotin and streptavidin, generating much larger fluorescent complexes. Thus, a further amplified FP signal was obtained. Under optimized conditions, the developed method exhibits a linear response to nucleic acid in the concentration range of 0–2.5 nM, and the limit of detection is 35 pM. Moreover, the FP assay also showed high precision, pronounced specificity and the applicability in complicated human serum matrix.

1. Introduction

Among numerous optical techniques, fluorescence polarization (FP) has been gathered much attention due to its fast, homogeneous format, accuracy, and automated high-throughput capability [1]. In general, FP is inversely proportional to the rate of rotation and tumbling of a molecule. A key factor influencing the FP value is the molar mass of the molecule or complex being measured [2]. That is, the rate of a small fluorescent molecule rotation in solution is fast, and the corresponding FP value is small. But the FP value will increase if the fluorescent molecule forms a complex with another molecule. The relationship between the molecular mass of the fluorescent molecule and its FP value makes polarization to be an ideal technique for the investigation of biomolecular interactions. Recent years, several reports using mass amplifiers have been developed for FP assay to improve the assay performance. For example, Peyrin and co-workers [3] used a single-strand binding (SSB) protein as a signal enhancer for the FP assay of small molecules. Yang and co-workers [4] described a graphene-aided amplified strategy to detect the ATP molecule. Although these strategies exhibit high sensitivity, the involved turn-off approaches may induce false positive signals since the disassembly process could result from nonspecific protein binding in real samples. To overcome this limitation, developing turn-on FP detection methods with high sensitivity and selectivity becomes a necessary topic for analysts.

The hybridization chain reaction (HCR) based on toehold-mediated DNA strand displacement is a simple and effective approach for signal amplification [5,6]. An important distinction of HCR amplification to other methods is the fact that specific DNA fragments are not amplified but rather serve as initiator molecules to trigger self-assembly of oligonucleotide nanostructures. In particular, two stable species of hairpins can be opened only in the presence of the initiator, which can trigger a cascade of hybridization events to yield nicked double helices analogous to alternating copolymers. This strategy not only provides efficient nonezymatic signal amplification, but also involves a unique assembly process which works under mild conditions. Nowadays, many studies combined the amplification capability of HCR with various sensing platforms and showed also good results in cell media [7], human serum [8], in vitro, or even in vivo [9,10].

* Corresponding authors. Tel.: +86 773 5845973; fax: +86 773 5832294.
E-mail addresses: jzhao12@163.com (J. Zhao), zhaoshulin001@163.com (S. Zhao).

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Nanoparticles have been widely used in many sensing systems because of their particular features, such as optical [11], magnetic [12], size-dependent physical and chemical properties [13]. Recently, some groups reported that nanoparticles could act as excellent mass amplifiers to enhance the FP signal [14,15]. In this work, SiO_2 nanoparticle modified with streptavidin (SA-SiNPs) was chosen as a model of nanoparticle amplifier. And combining with HCR, a double amplified FP assay was constructed. The biotin–streptavidin pairing has strong binding affinity, exceptional specificity and stability of the conjugate. The addition of target nucleic acid triggered HCR amplification, thus the HCR products and SA-SiNPs would form complex structures via specific biotin–streptavidin interactions which results in obvious amplification of FP value. The proposed FP assay exhibits a response to target, which can increase the sensing reliability and reduce the risk of false hits. Moreover, this FP assay has been successfully applied in human serum for DNA detection because of its resistance to environmental interferences. To the best of our knowledge, this is the first time to use nanoparticles combining HCR strategy for amplified FP detection.

2. Experimental

2.1. Materials and instrumentation

All oligonucleotides were obtained and HPLC-purified by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The DNA sequences [5,7,16] used in this work are shown in Table 1. Streptavidin (SA), tetraethyl orthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTES) and 1-ethyl-3(3-1-ethyl-3(3-dimethylaminopropyl)) carbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich (St. Louis, MO). Human blood samples were kindly provided by the No. 5 People's Hospital (Guilin, China). All other chemicals used in this work were of analytical grade. Water was purified with a Milli-Q purification system (Millipore Corp., Bedford, MA) and used throughout the work. Fluorescence measurements were carried out at room temperature on a LS 55 Fluorescence Spectrometer (PerkinElmer, USA). The FP signal was monitored at 520 nm with the excitation of 480 nm, and slits for both the excitation and the emission were set at 10 nm.

2.2. Synthesize and modification of SiO_2 nanaoparticles

The SiO_2 NPs were synthesized according to previous reported method [17] and modified slightly. In brief, 1.77 mL of Triton X-100, 7.5 mL of cyclohexane, 1.8 mL of 1-hexanol and 420 μL of water were mixed and stirred continuously followed by the addition of 100 μL of TEOS 15 min later. After that, 60 μL of NH_4OH was added to initiate silica polymerization. After 24 h, 50 μL of TEOS, 25 μL of APTES and 30 μL of NH_4OH were added and stirred for 16 h. Finally, the obtained amine-modified SiO_2 NPs were centrifuged and washed with ethanol four times. The prepared SiO_2 NPs were characterized by transmission electron microscope (Fig. S1) and the size is about 60 nm.

1 mg amine-modified SiO_2 NPs were washed twice with MES buffer ($C_6H_{13}NO_4S$–NaOH, pH 6.0). After that, the SiO_2 nanoparticles were suspended in 25 μL MES buffer containing 1 mg EDC with slightly shaking. Then, 80 μL 1 mg/mL streptavidin was added into the mixed solution and incubated for 2 h at room temperature with gentle shaking. Excess streptavidin was removed from the SiO_2 nanoparticles solution by ultrafiltration with a centrifugal filter. After that, the streptavidin-coated nanoparticles (SA-SiNPs) were washed twice with 100 μL PBS buffer and resuspended in 500 μL of PBS buffer. The resulting solution was stored at 4 °C in the dark.

2.3. Fluorescence measurements

DNA hairpin probes H1 and H2 were dissolved in 20 mM Tris–HCl buffer solution (1 M NaCl, pH 7.5). The solution was heated to 95 °C for 5 min, and cooled slowly to room temperature before use. Different concentrations of target DNA solutions were mixed with 100 nM H1 and 100 nM H2 to make a 10 μL volume of reaction solution. Then, the resulting solution was incubated at 37 °C for 1 h. After that, appropriate volumes of SA-SiNPs and Tris–HCl buffer solutions were added into above solution. After incubation at 37 °C for another 1 h, the FP signal of the mixture solution was measured in a 200 μL quartz cuvette at room temperature.

2.4. Gel electrophoresis analysis

The gel electrophoresis analysis was carried out on 3.5% agarose gels containing 2 μL of ethidium bromide per 50 mL of gel volume and dissolved in 1× TBE buffer at room temperature. 10 μL different reaction products with loading buffer were added to each lane. Agarose gels were run at a constant potential of 100 V for 60 min and visualized under UV light.

3. Results and discussion

3.1. Principle of amplified FP assay

The principle of amplified FP assay is shown in Scheme 1. In this design, the hairpin probe H1 was modified with biotin and fluorescein (FAM) was labeled on hairpin probe H2. The introduction of target nucleic acid to the sensing system triggers the autonomous cross-opening of probes H1 and H2 via HCR to form long nicked double-strand polymers (dsDNA), slowing down the rotation rate of fluorescein by the formation long HCR products. Thus the FP value was increased through HCR process. After the introduction of streptavidin-modified SiO_2 NPs (SA-SiNPs), the HCR-produced dsDNA products would bind to SA-SiNPs via specific biotin–streptavidin interactions. One HCR product could bind with several nanoparticles, resulting in a large molecular weight of dsDNA-SiNPs complexes. Then, a further dramatic increase of FP value was induced. However, on the contrary, no HCR or combination can be obtained in the absence of target nucleic acid, and on obvious enhancement of FP value could be obtained. Thereupon, the designed HCR and nanoparticles combination based double amplification strategy may provide a sensitive FP assay for nucleic acid detection.

In order to prove the principle of the assay, the performances were tested under different conditions. Fig. 1 is the obtained FP signal with or without 2.5 nM target DNA at various analysis conditions. And the inset is the corresponding FP signal increment (ΔFP, $\Delta FP = FP - FP_0$, where FP is the signal in the presence of target, FP_0 is the blank signal in the absence of target) upon the addition of 2.5 nM target DNA at each condition. When adding target DNA into H1 and H2 contained solution, an increase of FP signal was obtained compared with that of no target DNA (column 1). However, in the absence of H1 probe, no obvious ΔFP value was observed regardless of the absence or presence of SA-SiNPs (columns 4 and 5, inset). This is because that the lack of H1 probe makes the HCR process impossible. The above results demonstrate that the target-triggered HCR process could induce an enhancement of FP signal. And this slightly small increment of FP signal might be because that the molecular weight of formed HCR product was not large enough to induce a significant change in FP, and a similar result was also observed in the previous report [8]. When SA-SiNPs was introduced into the detection system, a larger of ΔFP value was achieved (column 2, inset) compared with that of HCR only (column 1, inset). This