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A novel fluorescent detection for PDGF-BB based on dsDNA-templated copper nanoparticles

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ABSTRACT

A novel method for the detection of PDGF-BB has been developed using double-strand DNA-copper nanoparticles (dsDNA-CuNPs) as fluorescent markers. This assay relies on the premise that the aptamer-based probe undergoes a conformational change upon binding with target protein, and subsequently triggers polymerization reaction to generate dsDNA. Then, the resultant dsDNA can be used as a template for the formation of CuNPs with high fluorescence. Under the optimized conditions, the proposed assay allowed sensitive and selective detection of PDGF-BB with a detection limit of 4 nmol/L. This possibly makes it an attractive platform for the detection of a variety of biomolecules whose aptamers undergo similar conformational change.

1. Introduction

The interface between nanomaterial sciences and biology attracts substantial research efforts due to the broad possible applications in bio-nanotechnology [1]. A prime example is the study of the interactions between noble metal nanoparticles and DNA. Noble metal nanoparticles typically consist of a limited number of metal atoms (*e.g.* Au, Ag, and Pt), which display size and excitation wavelength dependent photoluminescence behavior [2]. The unique properties of these nanoparticles, as well as their physical and electrical features, allow noble metal nanoparticles to be widely used in cellular imaging and chemical/biological detection [3]. Especially, oligonucleotide-templated nanoparticles have attracted an explosion of interest in biological analysis because of their facile synthesis, tunable fluorescence emission, high photostability, and suitability [4]. Lately, Mokhir and co-workers have utilized dsDNA as scaffolds for the synthesis of fluorescent copper nanoparticles (CuNPs) at low concentrations of $CuSO_4$, and the resultant CuNPs have excellent fluorescence, whereas ssDNA do not support nanoparticle formation [5]. The specificity of CuNPs toward the dsDNA template makes it suitable for being used as a novel fluorescent marker. Although still in its infancy [6], such promising fluorescent CuNPs have drawn increasing attention in biosensing due to their ease of synthesis, low toxicity, outstanding spectral and photophysical properties. Therefore, the design and construction of biosensors using CuNPs as fluorescent markers for the detection of analytes is still of significant importance.

Platelet derived growth factor (PDGF), a growth factor protein found in human platelets, has increased in importance due to its role in the regulation of cell growth and division [7]. A PDGF dimer composed of two different types of disulfide-linked polypeptide chains, designated A and B, occurs in three isoforms: PDGF-BB, PDGF-AB and PDGF-AA [8]. Among these isoforms, PDGF-BB is known to be directly implicated in the cell transformation process and in tumor growth and progression [9]. It is expressed at undetectable or low levels in normal cells but is found to be overexpressed in human malignant tumors [10]. As a potential protein marker [11], sensitive and rapid detection of PDGF-BB is particularly important in early cancer diagnosis, treatment, and prognosis of cancers. During the past years, various commonly antibody-based immunoassays have been developed for PDGF detection [12]. Unfortunately, these methods suffer from complexity, poor repeatability, and long assay times involving multiple washing steps, thereby limiting its usefulness. Recently, several new approaches by employing aptamers as recognition elements have been developed, including fluorescence, colorimetry and electrochemistry techniques [10b,13]. However, these methods require labeled aptamers and the use of expensive fluorophores or coupling reagents. In addition, the precise target binding sites and

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the resulting conformational changes of the aptamers are generally uncertain, so the design of labeling strategies is not always easy [14]. Moreover, there is a concern that the conjugation of a functional group such as fluorophore to an aptamer will ultimately weaken the affinity of the aptamer toward its target [15]. Therefore, the development of simple and label-free methods for aptamer-based detection of protein has gained increasing attention recently.

Herein, we reported a simple, low cost method to detect PDGF-BB by using novel fluorescent markers of dsDNA-CuNPs. In the presence of the target protein, a structure-switching probe designed from the original aptamer can be folded into its stable aptamer-protein complex and initiate polymerization to generate a stable dsDNA. Then, the produced dsDNA can be used as a template for the formation of CuNPs through the reduction of Cu^{2+} by ascorbate, and thereby, the formed CuNPs show high fluorescence. In comparison with a number of previous methods, this approach avoids the usage of labeled aptamers, or the necessity to design complicated fluorescent aptasensors. More importantly, the highly specific interactions between the aptamer and PDGF-BB provide high selectivity, and the strongly fluorescent dsDNA-CuNPs markers offer high sensitivity (LOD 4 nmol/L) for the detection of PDGF-BB, which is comparable to those previously reported aptasensors [10b,13c]. In addition, this design is merely based on nucleic acid hybridization and polymerase replication, so it can generally be applied to other aptamers for the detection of a large number of biomolecules.

2. Experimental

Oligonucleotides used in this work were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China), with their sequences listed in Table 1. Isoforms PDGF-AA, PDGF-AB, and PDGF-BB were purchased from PeproTech Inc. (New Jersey, USA). Human IgG, bovine serum albumin (BSA) and human serum albumin (HSA) were obtained from Dingguo Biochemical Reagents Company (Beijing, China). Myoglobin (MYO) was purchased from Abcam Company (Massachusetts, USA) and C reactive protein (CRP) was purchased from Biovision Inc. (California, USA). All reagents were prepared and handled according to the specifications of the supplier. The deoxynucleotide triphosphates (dNTPs) were obtained from Sangon Biotechnology Co. Ltd. and Klenow fragment polymerase ($3' \rightarrow 5'$ exo-, KF polymerase) was purchased from New England Biolabs Inc. (Massachusetts, USA). Sodium ascorbate was obtained from Sigma–Aldrich (Shanghai, China). All work solutions were 10 mmol/L MOPS (150 mmol/L NaCl, 1 mmol/L $MgCl_2$, pH 7.5). All solutions were prepared with Milli-Q water (18.2 MΩ cm) from a Millipore system.

Fluorescence measurements were carried out on a Hitachi F-4500 fluorescence spectrometer (Hitachi Ltd., Japan) using a square quartz cuvette with an optical path length of 1.0 cm. Excitation and emission slits were all set for a 10.0 nm band-pass. The excitation wavelength was set at 340 nm, and the emission spectra were collected from 520 nm to 660 nm. The fluorescence intensity at 575 nm was used to evaluate the performances of the proposed assay strategy. All measurements were carried out at room temperature unless stated otherwise.

Table 1
Oligonucleotides designed in the present study.[a]

Oligonucleotide	Sequence (5′–3′)
Probe DNA	TAC TCA TAC GCT CAT ACG TTC ATC ACG ACT ACG TTT CAC ACA GGC TAC GGC ACG TAG AGC ATC ACC ATG ATC CTG TGT
Blocker DNA-12	GTG TGA AAC TCT
Blocker DNA-13	TGT GTG AAA CTC T
Blocker DNA-14	CTG TGT GAA ACT CT
Blocker DNA-15	CCT GTG TGA AAC TCT
Blocker DNA-16	GCC TGT GTG AAA CTC T
Blocker DNA-17	AGC CTG TGT GAA ACT CT
Blocker DNA-18	TAG CCT GTG TGA AAC TCT

[a] The underlined regions of the probe indicate anti-PDGF-BB aptamer.

2.1. Procedure for PDGF-BB detection

A number of 200 μL solutions consisting of 10 mmol/L MOPS (150 mmol/L NaCl, 1 mmol/L $MgCl_2$, pH 7.5), probe DNA (200 nmol/L), blocker DNA (200 nmol/L) and different concentrations of PDGF-BB were prepared, and incubated at 37 °C for 15 min. Then, 180 μL of the incubated solution was added to 20 μL of polymerization reaction buffer containing 10 mmol/L MOPS, dNTPs (3 mmol/L), KF polymerase (5 units). The polymerization reaction was carried out at 37 °C for 30 min. Subsequently, the resulting solution was incubated at 75 °C for 20 min to inactivate the KF polymerase. After the resulting solution was cooled to ambient temperature, 1 μL of ascorbate (200 mmol/L) and 1 μL of $CuSO_4$ (10 mmol/L) were added to 98 μL of the resulting solution and allowed to incubate for 10 min *prior to* the fluorescence measurement.

2.2. Gel electrophoresis

Analysis by electrophoresis was carried out on 12% non-denaturating polyacrylamide gel electrophoresis (PAGE), cast and ran in 1× TBE buffer (90 mmol/L Tris, 90 mmol/L boric acid, 2 mmol/L EDTA, pH 7.9) at room temperature. Electrophoresis was performed at a constant potential of 100 V for 90 min with loading of 10 μL of each sample into the lanes. The resulting gel was stained by SYBR Gold, and analyzed using Gel Imaging System (Tanon 2500, Tianneng Ltd., Shanghai, China).

3. Results and discussion

3.1. Design and feasibility of dsDNA-CuNPs based PDGF-BB assay

The design of PDGF-BB detection based on dsDNA-CuNPs as fluorescent markers is shown in Fig. 1. The assay is composed of two nucleic acid strands (probe DNA and blocker DNA). The probe DNA consists of two regions: Region I includes the aptamer region for PDGF-BB and region II consists of a sequence that can serve as the template for polymerases. The probe DNA is blocked by a blocker DNA (3′ end with 3 base noncomplementary to the probe) to prevent it from uncontrolled folding into an active configuration in the absence of targets. Addition of PDGF-BB to the system separates the blocker DNA from the probe DNA, making the probe DNA fold into an aptamer-protein complex that includes a 7-base duplex structure (step 1). The 3′ end of the probe DNA can function as a primer to initiate a polymerization reaction in the presence of Klenow fragment polymerase and dNTPs, yielding a double-stranded DNA duplex (step 2). Then, the produced dsDNA can act as an efficient template for the formation of CuNPs (step 3), and the formed CuNPs show high fluorescence (Fig. 2, curve a). However, in the absence of PDGF-BB, the probe DNA and blocker DNA could not initiate polymerization reaction to form the dsDNA. Due to the absence of dsDNA, the CuNPs would practically not be formed. Accordingly, low fluorescence was observed (Fig. 2, curve b). Control experiments proved that neither probe DNA nor blocker DNA could be used as an effective template for the formation of CuNPs, and no fluorescent signal could be detected (Fig. 2, curves c and d).