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Short communication

Aptamer-based colorimetric detection of proteins using a branched DNA cascade amplification strategy and unmodified gold nanoparticles

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ABSTRACT

A branched DNA amplification strategy was employed to design a colorimetric aptameric biosensor using unmodified gold nanoparticles (AuNPs). First, a programmed DNA dendritic nanostructure was formed using two double-stranded substrate DNAs and two single-stranded auxiliary DNAs as assembly components via a target-assisted cascade amplification reaction, and it was then captured by DNA sensing probe-stabilized AuNPs. The release of sensing probes from AuNPs led to the formation of unstable AuNPs, promoting salt-induced aggregation. By integrating the signal amplification capacity of the branched DNA cascade reaction and unmodified AuNPs as a sensing indicator, this amplified colorimetric sensing strategy allows protein detection with high sensitivity (at the femtomole level) and selectivity. The limit of detection of this approach for VEGF was lower than those of other aptamer-based detection methods. Moreover, this assay provides modification-free and enzyme-free protein detection without sophisticated instrumentation and might be generally applicable to the detection of other protein targets in the future.

1. Introduction

Aptamers, like antibodies, have high-affinity binding interactions toward target molecules with great specificity. Therefore, aptamers are considered alternative recognition elements to monoclonal antibodies and are used extensively as sensors and diagnostic agents (Chang et al., 2011; Kim et al., 2014; Ma et al., 2015). A number of aptamer-based sensing systems, so-called aptasensors, have been reported, including electrochemistry (Xie et al., 2014), surface plasmon resonance (Chang et al., 2012b), fluorescence systems (Zhao et al., 2014), colorimetric assays (Chang et al., 2014a), and quartz crystal microbalance (Song et al., 2014). Despite the attractive sensitivity of these methods, most require the immobilization of DNA aptamers on the sensing surface or fluorescence labeling, which are usually laborious and time-consuming. In addition, the expensive synthesis of thiol-modified or fluorescence-labeled probes increases the cost of detection. As an alternative approach toward the development of label-free and immobilization-free aptasensors, unmodified gold nanoparticle (AuNP)-based colorimetric strategies have received intensive attention during the last 10 years owing to their simplicity and ease of operation (Li and Rothberg, 2004). This colorimetric sensing method takes advantage of the red-to-blue color change that arises from surface plasmon coupling during AuNP aggregation and can readily be seen by the naked eye (Saha et al., 2012). Typically, AuNPs were originally stabilized with unstructured target-binding aptamers. Upon binding to their target, aptamers fold into unique structures that switch off from AuNP surfaces, resulting in AuNP aggregation (Liu, 2012). Recently, we developed a signal-on architecture for aptamer-based sensors via a target-induced structure-switching DNA aptamer (Chang et al., 2013, 2014b). Although these colorimetric approaches are simple, their sensitivity is still unsatisfactory. A major limitation of previous label-free AuNP sensors is that each target interacts with only a single DNA probe on AuNP surfaces, thus hindering the

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sensing performance. As such, a simple and straightforward amplified approach for label-free AuNP-based aptasensing still needs to be developed.

Recently, interest in isothermal nucleic acid amplification has grown as an efficient way to amplify biosensing events (Chang et al., 2012a; Jung and Ellington, 2014; Li and Macdonald, 2015). Various approaches, including enzyme-assisted signal amplification strategies and enzyme-free signal cascades, have been employed for DNA or other targets using aptamers as the molecular recognition unit (Wang et al., 2014). Among the signal amplification methods, hybridization chain reaction (HCR), in which one target molecule leads to a chain reaction of recognition and hybridization events between a pair of complementary, kinetically trapped hairpins, has attracted considerable attention (Huang et al., 2011). A prominent merit of HCR over other competing amplification assays is that it allows for specific self-assembly and extension at room temperature without the involvement of enzymes. Nevertheless, unlike exponential amplification methods, such as polymerase chain reaction, the current form of HCR provides linear DNA self-assembly reaction kinetics (Dirks and Pierce, 2004). In addition, the reaction is slow; several hours are required to obtain the final signal, which is unfavorable for point-of-care diagnostics (Spiga et al., 2014; Wang et al., 2015). Lately, Xuan and Hsing (2014) developed a nonlinear HCR (NLHCR) system that provides exponential growth of branched DNA nanostructures after initiation via the DNA trigger. It has distinct advantages over the conventional HCR procedure, including a higher amplification efficiency and more rapid amplification kinetics. Although this approach is designed for DNA sensing within 30 min, it is expensive to modify single-stranded DNA with fluorophores. Additionally, its application is currently limited to nucleic acid analysis and it has not been validated as a signal amplification strategy for the design of AuNP-based colorimetric biosensors.

Herein, a label-free colorimetric aptasensor system was developed and integrated with the NLHCR strategy to achieve a sensitive protein assay. Vascular endothelial growth factor (VEGF), an important protein for angiogenesis and vascular permeabilization, was selected as a model protein (Otrock et al., 2007). By using this colorimetric assay, as few as 3.7 fmole of VEGF could be detected within an hour.

2. Experimental

2.1. Colorimetric assay using citrate-capped AuNPs

Prior to measurements, S1 and S2 were formed separately by heating mixtures of equimolar solutions (4 μM) of the a strand and b strand for 5 min and cooling to room temperature for 1 h before use. In the first step, target proteins were mixed with aptamer hairpin probes in phosphate-buffered saline (PBS) buffer solution (40 μL) and then incubated for 10 min (solution A). Afterward, equimolar solutions (4 μM) of S1, S2, A1, and A2 were added to the mixture solution (protein/hairpin probe) (solution B). After incubation for 25 min, 50 μL of solution B was added to an AuNP solution of 150 μL (solution C). After 10 min, solution C was monitored by UV–vis characterization.

2.2. Colorimetric assay using DNA sensing probe-stabilized AuNPs

First, solution A and solution B were prepared as previously described. Simultaneously, 10 μL of 6 μM DNA sensing probe was added to 140 μL of AuNP solution (solution D). After incubation of solution B for 25 min, 50 μL of solution B was added to 150 μL of solution D (solution E). Next, solution E was reacted for 10 min and 1 μL of NaCl (2 M) was added to the reaction mixture for 5 min.

For other details please see Supplementary materials.

3. Results and discussion

3.1. Colorimetric assay using citrate-capped AuNPs

The principle of the branched DNA cascade amplification-based aptamer detection method is illustrated in Scheme 1. This approach consists of five main components: a bifunctional hairpin probe (HP in Scheme 1), two double-stranded substrate DNAs (S1 and S2), and two single-stranded auxiliary DNAs (A1 and A2). The bifunctional hairpin probe consists of the VEGF aptamer sequence (blue) and the initiation sequence to trigger the NLHCR amplification reaction (yellow). Part of the initiation sequence is specially designed to be complementary to the VEGF binding aptamer sequence in an initially locked format. This probe is employed as a bridge to connect the aptamer sensing module and the NLHCR amplification module. In the absence of VEGF, the aptamer probe exists predominantly as a hairpin-folded structure at equilibrium, and the amplification reaction does not occur; it is 'closed' for NLHCR. Unlike traditional double-stranded DNAs with a duplex structure, the substrate DNAs with a single-stranded toehold domain (5 nucleotide (nt); strand a for S1 and S2) are designed to have the unpaired domains in the middle, which result in bulge-loop structures. To avoid spontaneous opening of substrate DNAs ascribed to the breath of each end of the substrates (Jiang et al., 2014), the bulged loop sequences of S1 and S2 were both designed to have 7 nt longer than the toehold domain. Additionally, auxiliary DNAs are designed to have no additional secondary structure because the presence of secondary structure in the single-stranded auxiliary DNAs could slow the process of DNA branch migration exceedingly (Zhang and Seelig, 2011). Predicted secondary structures of S1, S2, and HP were examined using NUPACK in Fig. S1. Thus, the substrate DNAs and hairpin probe stay metastable and can coexist with auxiliary DNAs until triggered by protein targets.

After the addition of AuNPs, single-stranded DNA (A1 and A2) is adsorbed by citrate-capped AuNPs and the stability of AuNPs increases, resulting in AuNP dispersion. In the presence of VEGF, it binds to the corresponding aptamer sequence and disturbs intramolecular DNA hybridization to open the hairpin structure. Therefore, conformation rearrangement of the hairpin aptamer probe results in a switch to the 'activated' initiation sequence, and thereby acts as a trigger for a cascade amplification reaction of NLHCR. In the initial step of NLHCR, partial strand displacement of S1 is initiated by toehold hybridization with the initiation sequence of the aptamer probe on the S1a strand and occurs via the migration of the initiation sequence at the 5' end. Then, A1 binds to the newly exposed toehold domain of the S1b strand, and the double-stranded byproduct B1 is released from S1. The brown domain of S2a serves as a toehold for interactions with the unstructured region of S1a, resulting in the formation of a two-branch DNA structure. After the introduction of A2, the two-branch DNA is separated to release the byproduct B2 via A2-assisted strand displacement reactions. Additionally, A2 provides two new initiation sequences to which S1 binds to initiate a new cycle of the amplification reaction. Therefore, dendrimer-like DNA is continuously constructed by the chain reaction. After incubation with the AuNP solution, the steric hindrance and complex conformation of dsDNA (Hussain et al., 2013; Shawky et al., 2010) result in ineffective adsorption of the DNA dendrimer on AuNPs, which makes AuNPs susceptible to salt-induced aggregation. In the absence of VEGF, DNA amplification components (i.e., substrate DNA and auxiliary DNA) exist stably so that nearly no amplification is triggered. Accordingly, auxiliary DNA in its single-stranded form can render the AuNPs stable against color changes.