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Designed diblock hairpin probes for the nonenzymatic and label-free detection of nucleic acid

Junlin Wen [a,b,c], Junhua Chen [b,*], Li Zhuang [b], Shungui Zhou [b,*]

[a] Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, China
[b] Guangdong Key Laboratory of Agricultural Environment Pollution Integrated Control, Guangdong Institute of Eco-Environmental and Soil Sciences, Guangzhou 510650, China
[c] University of Chinese Academy of Sciences, Beijing, China

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ABSTRACT

The detection of nucleic acid sequences is of great importance in a variety of fields. An ultrasensitive DNA sensing platform is constructed using elaborately designed diblock hairpin probes (DHPs) that are composed of hairpin and poly-adenine blocks. The introduction of an initiator DNA target triggers the catalytic assembly of probes DHP1, DHP2 and DHP3 to fabricate numerous poly-adenine-tailed branched DNA junctions, which significantly amplify the signal of the target-DNA-recognizing event without any enzyme. Coupled to a gold nanoparticle-based colorimetric assay, the amplified recognition signal can be quantitatively detected or visually read with the naked eye. The combination of the high-efficiency target-catalyzed DHP assembly and sensitive gold-based colorimetric assay offers an ultrasensitive detection of DNA with a detection limit of 0.1 pM and a dynamic range from 0.01 to 5 pM. The proposed sensing platform can discriminate even single-base mutations. Moreover, the sensing platform can be expanded to detect pollutant-degrading-bacteria-specific DNA sequences. The proposed sensing system should offer an alternative approach for the detection of nucleic acids in the fields of microbiology, biogeochemistry, and environmental sciences.

1. Introduction

Sequence-specific methods for detecting nucleic acids are critical for medical diagnostics (Jung and Ellington, 2014), food safety (Kim et al., 2014) and environmental science (Tadmor et al., 2011). Many detection systems have been developed by making use of enzymes as amplifiers because of the low abundance of targets in natural environments. Amplificatory tools, such as polymerase (Manrao et al., 2012), ligase (Shen et al., 2012; Wee et al., 2012) and exonuclease (Ju et al., 2012; Xuan et al., 2012), in DNA detection offer exquisite sensitivity and are commonly used to follow target–probe hybridization. However, these enzyme-based assays are labor-intensive, require complex operation, and have a relatively high cost. In contrast, catalytic hairpin assembly (CHA) is a simple and enzyme-free amplifying strategy that can effectively amplify and transduce the signal from DNA hybridizing events (Yin et al., 2008; Jiang et al., 2013b; Chen et al., 2015b). The CHA employs the target-initiated circular assembly of metastable hairpin probes to fabricate branched DNA junctions. Its feasibility to encode DNA functional and structural information, together with catalytic amplification with negligible background, offers unique features for point-of-case analyte detection. In fact, CHA has been used to transduce analyte-recognizing event to a variety of detection modalities, such as electrochemical chemiluminescence (Chen et al., 2015a), electrochemistry (Zhang et al., 2015) and photo-luminescence (Fu et al., 2013). Although these detection techniques are sensitive, their requirements for advanced instrumentations have to some extent limited the field applications.

Gold nanoparticle (AuNP)-based colorimetric detection techniques require simple instrumentation and can be easily read with the naked eye. AuNP colorimetric assays, when coupled with CHA, have seen significant applications in the detection of DNA sequences because target hybridization can be easily transformed to color changes (from red to blue). These assays are commonly realized by modifying a thiolated DNA probe on the surface of AuNPs through the well-established strong Au–S chemistry such that target–probe cross-linking induces their aggregation (Deng et al., 2012; Su et al., 2015). However, cross-link-based methods frequently suffer from being too time consuming (20–40 h) for the preparation of AuNP–DNA conjugates (Xia et al., 2010) due to the tedious process of Au–S chemistry-dependent modification.

* Corresponding authors.
E-mail addresses: jhchen@soil.gd.cn (J. Chen), sgzhou@soil.gd.cn (S. Zhou).

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Moreover, these methods have relatively poor detection limits (low nanomolar). Poly adenine (polyA), a recently reported linker between AuNPs and DNA, has shown a similar affinity to the thiol group (Jiang et al., 2013a; Chen et al., 2014). In addition, polyA requires no modification during synthesis, reducing the cost of detection. Moreover, polyA-mediated binding could be easily modulated (Zhang et al., 2012) and in fact has been used for the preparation of DNA–AuNP conjugates (Pei et al., 2012). However, polyA has not been exploited for the design of a diblock hairpin probe, coupling to CHA reaction, for the development of a nonenzymatic and label-free nucleic acid assay.

In this work, novel diblock hairpin probes composed of hairpin and polyA blocks were elaborately designed. In the presence of target DNA, the designed probes (DHP1, DHP2 and DHP3) are able to construct branched DNA junctions that possess tails of polyA, which can bind on the surface of AuNPs and aggregate them, triggering a color change in the colloidal gold solution. Based on these versatile probes, a nonenzymatic and label-free sensing platform was developed to detect DNA for the first time. Due to the high efficiency of target-triggered catalytic diblock hairpin probe assembly and the sensitive AuNP-based colorimetric assay, a significantly enhanced sensitivity was achieved. Furthermore, the proposed platform exhibited high specificity and was capable of identifying a target from single base mutations. The proposed detection system provides an ultrasensitive sensing platform for the detection of nucleic acid.

2. Materials and methods

2.1. Reagents and materials

The oligonucleotides were synthesized and purified by Sangon Biotech Co. (Shanghai, China), and the sequences are presented in Table S1. Chloroauric acid ($HAuCl_4$) and trisodium citrate were purchased from Sigma-Aldrich (St. Louis, MO). Other common regents were used as received. All of the solutions were prepared using ultra-pure quality water (18.2 MΩ cm resistivity) obtained from a Milli-Q Water System (Millipore Corporation, Bedford, USA).

2.2. Synthesis of gold nanoparticles

AuNPs (15 nm) were prepared by the typical citrate reduction of $HAuCl_4$ according to a previous report (Li and Rothberg, 2004). Briefly, a 5-mL aqueous solution of sodium citrate (1 wt%) was added to a boiling solution of $HAuCl_4$ (100 mL, 0.01 wt%). After the solution color changed to red, the reaction mixture was allowed to boil for 30 min. Then, the synthesized gold solution was cooled to room temperature and stored at 4 °C before use. All of the glassware that was used in the synthesis was cleaned with aqua regia and rinsed with pure water.

2.3. Gel electrophoresis

Lyophilized oligonucleotides were dissolved in ultra-pure water to prepare concentrated DNA stock solutions and later diluted to reaction conditions (50 mM Na_2HPO_4, 1 M NaCl, pH 6.8). The oligonucleotides (probes DHP1, DHP2 and DHP3) were heated to 95 °C for 5 min and then slowly cooled to room temperature and stored at 4 °C prior to use. The target DNA (200 nM) was mixed with 1 μM (final concentration) each of the annealed probes (DHP1, DHP2 and DHP3) and incubated at 25 °C for 2 h. A 3% agarose gel was prepared using TAE buffer (40 mM Tris AcOH and 2.0 mM Na_2EDTA, pH 8.5). The samples were first mixed with loading buffer (V/V, 1:5) and then added to the gel (5 μL per sample). After running at 120 V for 30 min, the gel was photographed in a Gel Doc XR+ system (Bio-Rad).

2.4. Transmission electron microscopy analysis (TEM)

A TEM analysis was performed according to a previous protocol (Deng et al., 2005). The samples that were used for the TEM analysis were prepared by dropping onto copper grids (10 μL per sample). After drying at room temperature, TEM images were taken with a Jeol JEM-2011 (Jeol Ltd., Japan) that was operated at 80 kV in bright-field mode.

2.5. Assay procedure

To begin, 32 μL of each 3-μM annealed hairpin probe and 224 μL of buffer (50 mM Na_2HPO_4 and 1 M NaCl, pH 6.8) were added in a 1.5-mL tube. After mixing with a pipettor, the mixture was aliquoted into eight separate tubes (36 μL per tube). To these tubes, 4 μL each of 50 pM, 30 pM, 10 pM, 5 pM, 3 pM, 1 pM, 0.5 pM and 0.1 pM target DNA was added to reach a total volume of 40 μL. These samples were then mixed and allowed to react for 2 h at room temperature. Subsequently, 30 μL of the prepared reaction mixture was added to an AuNP solution of 270 μL. After incubating for 3 min, the mixed solutions were measured using a TU-1900 UV–vis spectrometer (Beijing, China).

3. Results and discussion

3.1. Principle of the proposed sensing method

The designed DHPs comprise two concatenated domains: a polyA anchoring block and a functional hairpin block (Scheme 1a, left). The hairpin blocks of DHPs contain toeholds (a special nucleation site), denoted a, b and c. The relationships between the segments of DHPs are specified (Scheme 1a, right). In the absence of target DNA (initiator, I), the DHPs are kinetically impeded from forming branched junction, leaving the gold solution remained red (Scheme 1b, left). The assembly of branched DNA junctions occurs when initiator strand I, containing an exposed toehold a*, nucleates at the toehold of DHP1 and initiates a branch migration that opens the hairpin block, forming complex I/DHP1. The complex I/DHP1 has a newly exposed toehold b* that catalyzes the assembly of DHP2 to form I/DHP1/DHP2 and exposes toehold c*, which catalyzes the assembly of DHP3 to form I/DHP1/DHP2/DHP3. Following the above assembly steps, a disassembly step occurs, in which DHP3 replaces I from the complex I/DHP1/DHP2/DHP3, freeing I to initiate the additional assembly of branched junctions (Yin et al., 2008). The produced DNA junctions (DHP1/DHP2/DHP3) possess polyA tails that can combine multiple AuNPs and thereby aggregate them. This polyA-tailed DNA junction induced the aggregation of AuNPs, resulting in the color changing from red to blue (Scheme 1b, right). Because the color change is dynamically dependent on the concentration of target DNA, the proposed sensing system allows for the qualitative and quantitative detection of DNA.

3.2. Evaluation of the proposed sensing platform

The above mentioned mechanism was evaluated using target DNA as an analyte. Fig. 1a shows that color changes from red to blue in the presence of target DNA, whereas the control (without target) remains the characteristic wine red (inserted photo). This result is confirmed in the UV–vis absorption spectra, where the target sample displays absorption at 650 nm attributed to the surface plasmon resonance (SPR) absorption of aggregated AuNPs,