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An unmodified gold nanorods-based DNA colorimetric biosensor with enzyme-free hybridization chain reaction amplification

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ABSTRACT

A novel and simple colorimetric biosensor was developed by combining the unique optical properties of unmodified gold nanorods (AuNRs) with the amplification strategy of hybridization chain reaction (HCR), which was capable of detecting DNA sensitively and selectively. The detection mechanism is based on the dispersion/aggregation of AuNRs under the high concentration of salt. When target DNA introduces, the hybridization of hairpin DNA is triggered and a nicked double-helix DNA as HCR product forms which sticks tightly with AuNRs through a strong electrostatic adsorption and protects AuNRs from aggregation in high salt concentration condition. When no target DNA appears, AuNRs undergo aggregation due to the weak protection of hairpin DNA. The approach is able to detect target DNA in a range of 0–60 nM with a detection limit of 1.47 nM and exhibits high selectivity to distinguish fully matched and single-base mismatched DNA. Results demonstrate this method is label-free, modification-free, and enzyme-free, which holds great promise for routine sensing applications.

1. Introduction

DNA detection has attracted great attention in a variety of areas, including disease diagnosis [1], food security [2,3], environmental monitoring [4], and so forth. In the past decades, enormous efforts have been made for seeking DNA detection methods with relatively low cost, high sensitivity, and selectivity. However, current methods are mainly based on fluorescence [5], electrochemistry [6], chemiluminescence [7], and Raman spectroscopy [8], but usually require additional bio/chemical labels, complicated operations, and expensive instruments. Owing to the simple, cost-effective, and rapid responded characteristics, the colorimetric approach is ideal for DNA biosensing. The sensitivity is a key factor in DNA biosensing and a signal amplification process is always needed. As yet, diverse amplification strategies blossom for sensitive detection of DNA, whereas enzyme-based methods are limited due to the instability of enzyme and special reaction conditions. Alternatively, the enzyme-free amplification techniques such as hybridization chain reaction (HCR) and catalytic hairpin assembly (CHA), are charming and widely used for ultrasensitive detection of DNA. CHA-based amplification relies on the target recycle, in which a target triggers the hybridization first and when it finishes, the released target goes into another cycle [9–12]. However, the released target may cause a negative effect in nanoparticles-based colorimetric assay while HCR-based strategy is not. The concept of HCR was firstly raised by Dirks and Pierce in 2004, this system utilizes the competitive hybridization among nucleic acid chains to realize sensitive detection of DNA [13]. In a reaction solution, two pieces of coexisted hairpin DNA are stable and do not form into double-stranded DNA (dsDNA) until the initiator strands are added. Until now, numerous studies about HCR-based colorimetric detections have been reported and they establish three major classes of colorimetric assay based on HCR. The first one relies on the aggregation of metallic nanoparticles such as gold nanospheres (AuNSs) [14–18] and AgNPs [19]. The second one requires additional labels [20] or mimetic enzyme-catalyzed chromogenic reactions [21,22]. The last one possesses both two features above [23–25]. In the past years, the successfully developed HCR-based colorimetric detection assay have been sensitively detecting various analytes, including DNA [26], non-ionic small molecules [27], metal ions [28], and protein [29], holding great promise in routine sensing applications.

A colorimetric sensor is used for instantaneous analyzing through the absorbance change that even can be detected visually by color change. The signal-conversion element is extremely important to the design of the colorimetric sensor, nanomaterials like gold, silver, and copper play an important role in current sensor technology and especially gold-based nanomaterials are widely used. Gold nanomaterials

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possess diverse unique optical properties with surface-plasmon resonance (SPR) or localized SPR, resulting from the collective oscillation of free electrons when they resonate with incident light. The properties of SPR and localized SPR are highly depended on the material used and the size and shape of metallic nanoparticles involved [30]. The SPR phenomenon of gold nanoparticles behaving macroscopically is strong absorption bands displayed in the visible region and resulting in the origin of the observation of color. The absorption bands of gold nanoparticles are varied with the state of nanoparticles changing from dispersion to aggregation, meanwhile a change of absorbance and appearance color emerging. Therefore, gold nanoparticles are favorably employed in colorimetric assays.

Sphere-shaped gold nanomaterial, *i.e.* AuNSs, can be synthesized simply and has been extensively used in colorimetric detections. The AuNSs-based colorimetric systems are divided into two categories for DNA detection, modified and unmodified AuNSs. The modified AuNSs induce into aggregation by a sandwich formation [31], while unmodified AuNSs aggregate due to the release of the protectors from their surface [32,33]. Although AuNSs-based colorimetric biosensor has attracted great attention in DNA detection, the colorimetric biosensor based on gold nanorods (AuNRs) are rarely reported in particular for unmodified AuNRs. Unlike AuNSs, rod-shaped AuNRs are anisotropic and owe two different absorption bands, offering diverse potentials in biosensing. Unmodified AuNRs-based DNA colorimetric detection mainly dependents on the electrostatic interaction between positively charged AuNRs and negatively charged DNA [34,35]. Compared with single strand DNA, the formation of DNA duplex can greatly strengthen the electrostatic absorption between AuNRs and DNA, correspondingly an absorbance change raising as AuNRs were strongly adsorbed into an assembly [34,35]. To improve specificity, hairpin DNA is also utilized as a recognition probe without an amplification process, and consequently the sensitivity is still unsatisfactory [36]. Besides, the incorporation of HCR into AuNRs-based colorimetric bioassay has never been explored. Here, we first proposed a novel, simple, and highly specific DNA detection method by combination of AuNRs-based colorimetric detection with HCR-based amplification strategy. This detection method is label-free, modification-free, and enzyme-free and possesses superior specificity.

2. Experimental

2.1. Chemicals

Hydrogen tetrachloroaurate hydrate ($HAuCl_4{\cdot}3H_2O$), sodium borohydride ($NaBH_4$), ascorbic acid, and hexadecyltrimethylammonium bromide (CTAB) were purchased from Sigma-Aldrich (St Louis, MO, USA). Silver nitrate ($AgNO_3$) was purchased from Acros (Geel, Belgium). TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) and TAE buffer (2 M Tris AcOH, 100 mM Na_2EDTA) were purchased from Sangon Biotech Company, Ltd. (Shanghai, China). All other in analytical-graded reagents were used. The resistivity of Ultrapure water used in the experiments was 18.2 MΩ cm (Millipore, Billericay, USA). In the assay, the designed DNA sequences were synthesized by Sangon Biotech Company, Ltd. (Shanghai, China) and used a TE buffer to dissolve as concentrated DNA stock solutions. Then the DNA stock solutions were diluted using a sodium phosphate-sodium chloride buffer solution (50 mM Na_2HPO_4/0.5 M NaCl, pH 6.8). Table 1 lists all DNA sequences used in this study. The sequences of H1, H2, and I target were designed according to the previous report [13].

2.2. Gel electrophoresis

After 10 min heating at 95 °C, the H1 stock solution and H2 stock solution were then cooled down to 25 °C in 1 h. Target DNA with different concentration were added into a mixture of 1 μM each of H1 stock solution and H2 stock solution and incubated at 25 °C for 4 h. A 50 times diluted TAE buffer and an oligonucleotide dye, GoldView, were used to prepare the 3% agarose gel. Each of 10 μL samples were mixed with 2 μL the loading buffer. The gel was scanned by the gel image analysis system after running 1 h in the diluted TAE buffer with the voltage at 150 V.

2.3. Preparation of AuNRs

The AuNRs were chemically synthesized via a classical seed-mediated growth method according to previous reports [37,38]. Initially, a seed solution was obtained by quickly adding 0.6 mL of ice-cold $NaBH_4$ solution (10 mM, freshly prepared) into a mixture of 5 mL each of CTAB solution (200 mM) and $HAuCl_4$ solution (0.5 mM), after 2 min vigorous stirring the solution was aging for 1 h at 25 °C (water bath). As for the growth of AuNRs, 150 μL of $AgNO_3$ solution (4 mM) was firstly blended with a mixture of 5 mL each of CTAB solution (200 mM) and $HAuCl_4$ solution (1 mM), then adding 76 μL of ascorbic acid solution (79 mM) into above mixed solution and stirred gently for 20 s, after that the obtained solution was mixed with 12 μL of the above gold seeds solution and left at 25 °C for 2 h. Following that, the synthesized AuNRs was centrifuged (10,000 rpm, 15 min) to purify at 25 °C and the obtained pellet was resuspended in distilled water.

2.4. Measurement procedure

The classical target DNA detection step worked like this, after 10 min heating at 95 °C, the H1 stock solution and H2 stock solution were then cooled down to 25 °C in 1 h. Target DNA samples with different concentration were added into a sodium phosphate-sodium chloride buffer solution containing 250 nM H1 and H2, then incubated at 25 °C for 4 h. Following that, 80 μL of the washed AuNRs solution was mixed with 60 μL of the above-reacted solution and incubated at 25 °C for 10 min. Finally, 140 μL of the mixtures was sucked out for detection by UV–vis characterization.

2.5. Instrumentation

A SpectraMax M5 microtiter plate reader with Softmax® Pro 5.4 Software (Molecular Devices, Sunnyvale, USA) were applied to record UV–vis absorption spectra. Each well was added with 140 μL of the sample to obtain its absorption spectrum and the scanning wavelength for all measurements of AuNRs was set from 300 to 1000 nm. A JEM-1200EX transmission electron microscopy (TEM) was used for characterization of nanoparticles (JEOL, Tokyo, Japan). The preparation of TEM characterization sample was carried by fixing a carbon-coated copper grid with tweezers, and dropped 10 μL of the reaction solution on it then let it dry at room temperature. The ChemiDoc XRS + System was applied to scan the resulting gel electrophoresis images (Bio-Rad, Hercules, CA USA). The Zeta potential was performed under the following conditions: 25 °C of temperature, 90° of detector angle, incident laser wavelength at 633 nm by the Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK).

3. Results and discussion

3.1. Biosensing principle

As shown in Scheme 1, a sticky end was designed both for hairpin probes H1 and H2, for H1 at its 5′ end and for H2 at its 3′ end. These two hairpin probes are complementary to each other when opened. Otherwise, they are stable and sticking weakly to the surface of AuNRs by electrostatic absorption between DNA and AuNRs, but can't prevent AuNRs from salt-induced aggregation. When target DNA introduces, the hairpin H1 opens due to its sticky end pairs with the target DNA. As a result, a part of H1 is exposed and hybridizes with H2 at the sticky end also resulting the hairpin H2 opens, and the newly exposed part of H2