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Accurate and visual discrimination of single-base mismatch by utilization of binary DNA probes in gold nanoparticles-based biosensing strategy

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ABSTRACT

Herein we report a colorimetric biosensing strategy to discriminate single-nucleotide mutation in DNA with high selectivity using unmodified gold nanoparticles (AuNPs) as indicators. In the AuNPs-based colorimetric strategy, binary DNA probes were produced by splitting a long DNA probe in the middle for sensitive differentiation of single-base mismatch. The detection limit of this method toward target DNA was 5 nM. The developed system has superior advantages of utilization of inexpensive materials, simplicity and visualization. Moreover, binary DNA probes not only can distinguish single-base mutation in the target DNA very well, as compared to long DNA probe, but also can construct "AND" logic gate using two distinct target DNAs as inputs, which holds great potential for increasing the accuracy of disease diagnosis in clinical applications.

1. Introduction

Highly selective detection of DNA plays an important role in gene therapy, clinical diagnosis and mutation analysis [1–3]. In recent decades, several approaches such as fluorescence [4,5], luminescence [6], and colorimetry [7,8] have been adopted to analyze DNAs. However, these methods usually involve multi-step process and rely on complicated operation, expensive instrument, long detection time and various exogenous reagents, which increase the complexity, difficulty and cost of the detection.

Since Rothberg's group reported that single-stranded DNA (ssDNA) has much greater ability to prevent AuNPs from aggregation than double-stranded DNA (dsDNA) [9–11] AuNPs-based colorimetric methods were widely developed for DNA analysis because of their superior advantages, e.g. utilization of inexpensive materials, simplicity and visualization. But one of their major challenges is accurate recognition of single-base mismatches of long target DNA [12,13]. The selectivity of DNA hybridization is largely determined by the hybrid length between probes and target nucleic acid [14]. A long single-base mismatched target DNA cannot be distinguished well, since the produced long dsDNA hybrid is too stable to be separated easily at room temperature, particularly if the mismatch sites are located nearby the terminal of dsDNA [15].

Binary DNA probes have unique advantage in highly selective DNA recognition [16], and have been applied to many techniques for detection of nucleic acid, peptide and protein. Kolpashchikov's group put forward a theory that the under lying mechanism behind high selectivity of binary probes is that utilization of binary probes can decrease the free energy of probe-target dissociated state via increasing its entropy [17,18]. However, to the best of our knowledge, design of binary probe has not been introduced into unmodified AuNPs-based colorimetric system for DNA analysis.

Herein, we developed a new simple AuNPs-based colorimetric method for DNA detection and sensitive differentiation of single-base mismatches. Binary DNA probes were designed by splitting a long probe in the middle which is complementary to target DNA (Scheme 1). The sequences of DNAs used in this study are displayed in Table 1. Upon addition of target DNA to solution of binary DNA probes and 13 nm citrate-protected AuNPs, hybridization between target DNA and binary DNA probes greatly reduces the ability of protecting AuNPs from salt-induced aggregation, thus resulting in generation of blue color. Whereas the presence of single-base mismatch does not induce the AuNPs aggregation and the solution still exhibits red color. Accurate discrimination of single-base mismatch can be realized easily and visually using binary probes. In contrast to the long probe, the

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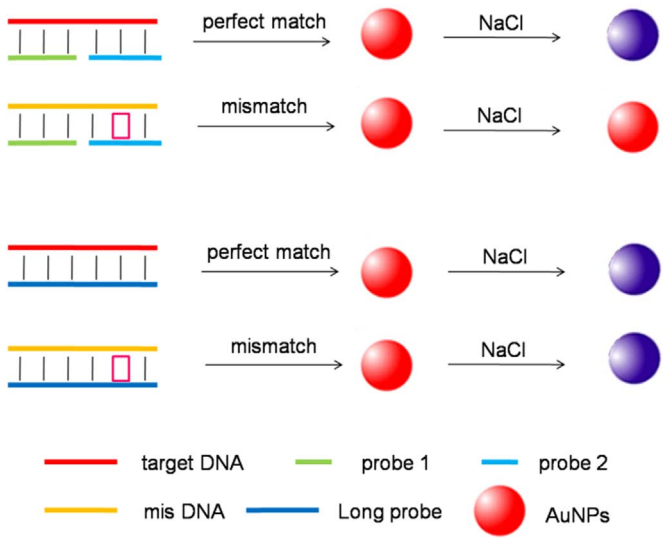

Scheme 1. Schematic illustration of AuNPs-based colorimetric system using binary DNA probes for sensitive discrimination of single-base mismatches. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article).

capability of our colorimetric method for differentiating single-base mismatch is significantly improved. What's more, compared with other AuNPs-based DNA detection methods, such as AuNPs dynamic light scattering [19], enzyme-assisted AuNPs colorimetry [13] and fluorescence [20] method, AuNPs/binary probes-based colorimetric strategy is enzyme-free, label-free and the detecting signal can be read out by naked-eye without complicated apparatus, which overcomes the detecting limitation of expensive materials and difficult operation.

2. Materials and methods

2.1. Materials

Chloroauric acid ($HAuCl_4$) was purchased from Sigma-Aldrich (Milwaukee, WI). Sodium citrate, magnesium chloride and sodium chloride were purchased from Beijing Chemical Reagent Company (Beijing, China). Tris (Hydroxymethyl) aminomethane (Tris) was purchased from Aladdin Inc. (Shanghai, China). Ultra-pure water (18.25 MΩ cm) was used throughout the experiments. DNA oligonucleotides were designed (Table 1) and bought from Sangon Biotech Co., Ltd. (Shanghai, China). All the DNA oligonucleotides were dissolved in Tris buffer (25 mM Tris-HCl, 200 mM NaCl, 1 mM $MgCl_2$, pH 7.8). The concentrations of oligonucleotides were determined by UV absorbance at 260 nm and respective extinction coefficient.

2.2. Instrumentation

Transmission electron microscopy (TEM) measurements were performed on a Hitachi H-800 transmission electron microscope (Hitachi, Japan) with accelerating voltage of 200 kV. The TEM samples were prepared by adding a drop of colloidal solution on a carbon-coated copper grid and allowing it to dry at room temperature. UV absorption spectra were obtained on a Cary 50 Scan UV–visible spectrophotometer (Varian, USA) at room temperature.

2.3. Synthesis of AuNPs

AuNPs (13 nm, 5 nM) were synthesized via a previously reported method [21]. 10 mL of 38.8 mM sodium citrate was rapidly added to 100 mL of 1 mM chloroauric acid which was vigorously stirred and heated to boiling. The solution was kept boiling for 10 min, stirred for another 15 min then cooled to room temperature and stored at 4 °C before use.

2.4. Colorimetric detection

For the detection of target DNA, DNA probe 1 and probe 2 with final concentration of 280 nM each were mixed with corresponding target DNA of final concentrations from 0 to 275 nM in buffer. The mixtures were heated to 90 °C and cooled down slowly to room temperature in 1 h to ensure that they were completely hybridized with each other. Then 2 μL of each mixture was added to 98 μL AuNPs solution and after a gentle shake for 5 min, 45 μL of 300 mM NaCl was added. Finally digital photographs and UV–vis spectra were collected. To investigate the selectivity of DNA binary probes, three single-base mismatched target (mis-11, mis-19 and mis-31) were tested using analogous procedures.

For the "AND" logic gate, two pairs of DNA probes were mixed together with inputs of different combinations. The final concentration of each DNA probe was 100 nM while DNA target was 40 nM. Other procedures were the same as those in the detection of one type of target DNA.

3. Results and discussion

Sodium citrate-capped AuNPs (particle size: 13 nm) has been widely used as colorimetric sensor in recent years for its optical properties [22,23]. Previous studies have proved that DNA adsorbs on AuNPs by electrostatic interaction, and the different electrostatic properties of ssDNA and dsDNA are the main reason of different binding force with AuNPs. The principles of different interactions of AuNPs between ssDNA and dsDNA and dramatic color change due to the aggregation of AuNPs have been thoroughly investigated. Moreover, 13 nm AuNPs can be prepared by a one-step synthesis only using $HAuCl_4$ and sodium citrate, which is very cheap, simple and timesaving [9]. In this paper, 13 nm AuNPs were used to construct AuNPs-based colorimetric biosensor in combination with the obtained binary DNA probes. Fig. 1 shows photograph and UV–vis spectra of AuNPs in the presence of binary probes (I1 and I2) with different concentrations of target I. In the absence of target I, the color of probes/AuNPs solution remained red. As the concentration of target DNA increased, AuNPs began to aggregate and the solution turned blue (Fig. 1A). The UV–vis spectra offered further evidence (Fig. 1B). As the concentration of target I increased, the characteristic surface plasmon resonance absorption band of AuNPs at 524 nm decreased, while an absorption band at 700 nm increased. The ratio of UV–vis absorption at 700 nm and 524 nm was calculated as ratiometric quantitative detection of target DNA. The linear relationship between the absorption ratio and the concentration of target DNA from 0 to 200 nM (linear equation: $A700/A524 = 0.167 + 0.005C_{targetI}$, $R^2 = 0.990$) was obtained (Fig. 1C), and the detection limit was 5 nM, which was comparable to those of many previously reported colorimetric DNA assays [9]. Transmission electron microscopy (TEM) images also confirmed that presence of target I induced the flocculation of AuNPs, then leading to the change of the solution color from red to blue (Fig. 2). These results validated that target I was quantitatively detected using the AuNPs-based colorimetric strategy. Fig. S1 (see Electronic Supplementary Material) displays the similar result of target IIdetection by virtue of this strategy.

Besides colorimetric detection of DNA, our AuNPs-based colorimetric strategy revealed remarkable universality that all the point mutations of target DNA could be well recognized wherever the mutated sites were located, which were derived from