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Colorimetric determination of DNA concentration and mismatches using hybridization-mediated growth of gold nanoparticle probes

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ABSTRACT

A novel colorimetric approach to sense DNA samples was demonstrated successfully based on a concept of hybridization-mediated growth of gold nanoparticles probes (AuNP probes). The growth size and conformation of the probes are dominated by DNA samples that hybridize with the probes. The growth probes serve as nanoparticle biosensors that can be utilized to analyze concentrations of DNA samples or to discriminate mismatches of DNA samples to the probes (complementarity of DNA) in terms of size- and shape-dependence optical properties. By means of the proposed method, not only the semi-quantitation of target DNA but also the differentiation of DNA mismatches can be readily achieved with a naked eye or simple spectrophotometer. This method features small consumption of reagents, a satisfactory detection limit (about 60 nM), a small duration of assay (a couple of minutes), and a simple procedure without thermal control.

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1. Introduction

Numerous authors have asserted that the diversity of individuals in an entire population is based on DNA variations in the human genome. Single-nucleotide polymorphism (SNP), the most abundant form of genetic variation, is of great significance, dominating the individuals who have differentiation in the risk of suffering from diseases, a susceptibility to diseases, and the response of their body to drug treatment [1,2]. The occurrence of some diseases, such as cancers [3], type-2 diabetes [4] and Alzheimer's disease [5] is strongly associated with SNP in correspondingly particular genes. Increasing evidence reveals that not only SNP (or single-point mutation), but also two- or multi-point mutations (multi-nucleotide polymorphism) pose certain diseases and individual differences [6–8]. A detection of these nucleotide polymorphisms and gene mutations is therefore imperative in both disease prescreening and the prediction of therapeutic response at an early stage.

Current approaches to identify DNA mutations aim mainly at SNP diagnosis with typical procedures including probe immobilization, labeling (with fluorescence or chemiluminescence), conjugation and reaction, sensing and readout (fluorescence analysis, gel electrophoresis, or mass spectra) [9–11]. Although these admirably sensitive means are widely used, they are labor-intensive, tedious and expensive because of costly detection systems. Through the prosperity of nanobio-technology, gold nanoparticles (AuNP) regarded as powerful probes in chemical, medical and biological sensing [12] are exploited in varied fashions to detect DNA [13–22], RNA [23], proteins [24–26], enzymes [27,28], peptides [29,30], metal ions [31–34], sulfite ion [35], small molecules [36,37], *Escherichia coli* [38] and the pH of a solution [39,40]. Various colorimetric assays for DNA detection are proposed and demonstrated [13–15,17–19,21,22] relying on size-dependent optical properties, great surface modifiability and biocompatibility of AuNP [12]. For most of them [13,15–17,21,22], a thermal process with temperature control is required to achieve satisfactory differentiation of DNA samples. A strategy that applied multicomponent AuNP probes to detect DNA was realized, but enzymatic modifications on AuNP are demanding [19]. An approach was reported to detect DNA based on a mechanism of non-cross-linking hybridization [14]; although DNA detection with this approach is rapid and easy with no thermal process, the applicable scope is limited as only some specific DNA, such as terminus-single-base mismatched DNA, can be detected. Another easy way was proposed to detect DNA using unmodified AuNP [18]; the detection thereby of target DNA fully complementary to probe DNA is adequate whereas that of DNA with a single base-pair mismatch to the probe is deficient.

Herein, we report a novel method for the visual and color detection of DNA mismatch in terms of a concept of hybridization-mediated growth of AuNP probes. This facile and expeditious

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method can be employed not only in semi-quantitative analysis of the given DNA (i.e. target DNA) but also in the identification of base-pair mismatches (single or multi-nucleotide polymorphism) of DNA samples, which is little investigated in published research but is indispensable for medical and genetic diagnosis.

2. Methods

2.1. Principle of colorimetric assay for DNA

Fig. 1 shows our proposed color assay for DNA detection based on a concept of hybridization-mediated growth of AuNP probes (please refer to the ESI for detailed experimental method and chemicals). Hydroxylamine (NH_2OH) reduces hydrogen tetrachloroaurate (III) ($HAuCl_4$) catalyzed by the surface of AuNP [41]; as a result, metallic gold deposits on the surface so as to develop the AuNP. In this work, 13-nm AuNP served as seeds for the growth. The unmodified single-strand DNA (ssDNA) attached to the gold surface is reported to affect the morphology of AuNP during the growth [42]. We stringently presume and manifest an idea that thiol-modified ssDNA or double-strand DNA (dsDNA) from the hybridization of the thiol-modified ssDNA with others on AuNP would profoundly influence the growth size and shape of the AuNP; the size and shape of AuNP would dominate its optical property. Information about DNA on the surface of AuNP could be therefore acquired on measuring the absorption curve of the growth of AuNP or on visually detecting the color of the growth AuNP solution.

The unmodified AuNP, after growth, extends to a diameter about 40 nm (top TEM photo), resulting in the solution becoming reddish. For AuNP modified with probe DNA, the AuNP probe after growth acquires a shape roughly spherical and size exceeding 50 nm (middle TEM photo), which is larger than unmodified AuNP. It is demonstrated that the structure of probe DNA like a scaffold supports and boosts the attachment of gold metal on AuNP. The resulting solution appears pink. In the presence of DNA fully complementary to a probe, CDNA, in a solution containing AuNP probes, CDNA hybridizes with the probe to form dsDNA on AuNP. After growth, the AuNP with dsDNA becomes much larger, and notably shows a mulberry- like shape of size about 80 nm (bottom TEM photo). This anomalous morphology is attributed to the dsDNA on AuNP; the resulting solution appears indigo. For the growth AuNP, not only the colors of their solutions but also the corresponding UV–vis absorption spectra are distinct.

2.2. Chemicals

Hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$, 99.9999%, Sigma–Aldrich) and hydrogen tetrachloroaurate(III) hydrate ($HAuCl_4{\cdot}3H_2O$, 99.999%, Sigma–Aldrich), all DNA samples and the probe with 5′-end labeled thiol (C6SH) (MDBio, Inc., Taiwan) and 13-nm AuNP solution (17 nM, TAN Bead Inc., Taiwan) were purchased from the indicated suppliers. Water (Milli-Q) was used to dissolve NH_2OH, $HAuCl_4$, DNA samples and the probe DNA. NH_2OH (400 mM) and $HAuCl_4$ (25.4 mM) solutions were prepared for the growth of AuNP (or AuNP probes). Probe DNA solution (100 μM) was prepared for the synthesis of AuNP probe; DNA sample solutions (1.0 μM) were prepared to test mismatches of DNA samples. DNA sample solutions and probe DNA solutions were stored in a freezer (−20 °C) when not in use.

2.3. Preparation of AuNP probe solution

The preparation of probe DNA-modified AuNP (AuNP probe) followed the literature procedure [13,42] with minor modifications. After the synthesis of AuNP probe, the concentration of the AuNP probe solution was modulated to 25 nM with water (Milli-Q) for ensuing use. The solution was stored in a refrigerator (4 °C) when not in use.

2.4. Growth of AuNP

For all experiments, AuNP probe (6 μL, 25 nM) was added to a DNA solution (350 μL) with the salt concentration 14 μM; various DNA concentrations were tested from 0 to 0.20 μM. DNA samples and AuNP probe underwent shaking incubation near 23 °C for 5 min. Afterward, NH_2OH (6 μL, 400 mM) and $HAuCl_4$ (6 μL, 25.4 mM) were successively added to the solution with rigorous blending. The growth of AuNP took approximately 30 s for completion.

2.5. Characterization

UV/Vis spectra were recorded with a spectrophotometer (NanoDrop 2000, Thermo Scientific, USA) with baseline correction. AuNP morphology was characterized with a FEG-TEM (200 kV, Tecnai™ G2 F20, FEI, USA). The distribution of particle size of AuNP was characterized with dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments Ltd., UK).

3. Results and discussion

3.1. Effect of CDNA concentration

After understanding that CDNA hybridizing with the probe has a crucial impact on the morphology of the growth AuNP, we investigated the effect of CDNA concentration on the size and shape of growth AuNP as well as the color of solutions. The color appearance of growth AuNP solutions obviously altered with varied CDNA concentration (Fig. 2a). Colors pink, amaranth, purple, dark purple, blue and indigo characterize CDNA concentrations 0, 0.03–0.06, 0.08, 0.11, 0.14, 0.17–0.20 μM, respectively. The variation of color of the solutions can provide a semi-quantitative interpretation of the CDNA concentration with a naked eye. The analysis of absorption spectra indicates that the surface-plasmon resonance (SPR) peak of the growth AuNP increases with increasing CDNA concentration, representing a red shift of the spectra from 535 nm at CDNA concentration 0 μM to 615 nm at CDNA concentration 0.20 μM (Fig. 2b). TEM results (Fig. 3) show that, with increased concentration of CDNA, the growth size of AuNP increased accompanied with a change of shape from roughly spherical to uneven (*mulberry-like*). The reason is that more CDNA hybridizing with the probe produces many dsDNA (CDNA-probe hybridization) occurring on the AuNP; the larger is the number of dsDNA, the bigger is the size and the more uneven is the shape of the growth AuNP.

As the CDNA concentration is increased from 0.20 to 0.56 μM, the color of a solution changes from indigo to blue or purple blue; the variation of the SPR peak implies a blue shift (Fig. 2). On analyzing the distribution of particle size of growth AuNP with dynamic light scattering (DLS) (Fig. 4), we discovered that, for CDNA concentration 0.56 μM, the size distribution is broad, revealing that the ratio of the number of small AuNP (sizes from 20 to 50 nm) to the total number of AuNP is larger than that for CDNA concentration 0.20 μM. Fig. 3d shows also that the size of the growth AuNP at CDNA concentration 0.56 μM is non-uniform. We hence infer that excessive CDNA are prone to attach on small gold seeds and facilitate their growth, thus producing such poor uniformity of growth AuNP; the color detections might become inaccurate for CDNA at a concentration exceeding 0.20 μM.

We depicted a correlation between the SPR peak of growth AuNP probe and the CDNA concentration as shown in Fig. 2c. A linear regression curve fitting the data possesses the *R*-square value 0.96, which represents that the proposed technique can serve to detect

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