Analytical Biochemistry 457 (2014) 19–23

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

A simple colorimetric DNA detection by target-induced hybridization chain reaction for isothermal signal amplification

Cuiping Ma [a,b], Wenshuo Wang [a], Ashok Mulchandani [b,*], Chao Shi [a,*]

[a] Shandong Provincial Key Laboratory of Biochemical Analysis, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, People's Republic of China
[b] Department of Chemical and Environmental Engineering, University of California, Riverside, CA 92521, USA

ARTICLE INFO

Article history:
Received 25 February 2014
Received in revised form 1 April 2014
Accepted 19 April 2014
Available online 26 April 2014

Keywords:
Gold nanoparticle
Colorimetric DNA detection
Hybridization chain reaction
Signal amplification
Nanobiosensor

ABSTRACT

A novel DNA detection method is presented based on a gold nanoparticle (AuNP) colorimetric assay and hybridization chain reaction (HCR). In this method, target DNA hybridized with probe DNA modified on AuNP, and triggered HCR. The resulting HCR products with a large number of negative charges significantly enhanced the stability of AuNPs, inhibiting aggregation of AuNPs at an elevated salt concentration. The approach was highly sensitive and selective. Using this enzyme-free and isothermal signal amplification method, we were able to detect target DNA at concentrations as low as 0.5 nM with the naked eye. Our method also has great potential for detecting other analytes, such as metal ions, proteins, and small molecules, if the target analytes could make HCR products attach to AuNPs.

Due to their high extinction coefficient and distance-dependent optical properties, gold nanoparticles (AuNPs)[1] have emerged as ideal materials for colorimetric biosensors [1–3]. The color of AuNPs is red in the dispersed state, but changes to purple or blue on aggregation because the surface plasmon band shifts to a longer wavelength [4]. As a result, AuNPs, especially DNA-functionalized AuNPs, have been successfully employed as a colorimetric probe for the detection of various analytes including nucleic acids [1,5–9], proteins [10], metal ions [11–13], and small molecules [14,15].

The aggregation of DNA-functionalized AuNPs is usually induced by the hybridization of DNA or the increase of salt concentration [16]. Mirkin and co-workers [17] first reported the crosslinked aggregation of AuNPs induced by DNA hybridization. However, the crosslinked AuNP aggregation induced by DNA hybridization is a relatively time-consuming process owing to steric considerations and electrostatic repulsive interactions [1]. Of note, steric considerations and electrostatic repulsive interactions provided by negatively charged DNA polymers enable DNA-functionalized AuNPs to remain stable even at relatively high salt concentrations [17]. Salt-induced aggregation is caused by the neutralization of the negative charges of DNA on AuNP surfaces [16,18]. Zhao et al. developed a simple and rapid colorimetric assay that exploited structure-switching DNA aptamers and the phenomenon of salt-induced AuNP aggregation [15]. In this assay, DNA strands attached to AuNPs were first hybridized with adenosine aptamer strands, which enhanced the stability of the AuNPs at a certain concentration of $MgCl_2$ by providing additional negative charges. Introduction of adenosine induced the switch of aptamer structure, and dissociated the aptamer strands from the AuNPs. The dissociation of aptamers decreased the salt stability of the AuNPs, which resulted in a rapid color change from red to purple.

In recent years, enzyme-free DNA circuits have attracted much attention [19]. The entropy-driven catalytic hybridization [20], triggered self-assembly [21,22], and see-saw gates [23] all can easily achieve signal amplification depending on the hybridization and strand-exchange reactions. Enzyme-free signal amplification is playing an important role in the development of biosensors and DNA nanotechnology. For instance, the hybridization chain reaction (HCR) [21,24], entropy-driven catalysis [25], and catalyzed hairpin assembly [7,26–28] have been effectively used for the design of biosensors. Moreover, DNA-based computation by strand displacement cascades has been reported [29,30].

In this work, we constructed a novel sandwich-like colorimetric system by combining DNA-functionalized AuNPs with HCR. In the system, the 3′ end of the target DNA strand hybridized with the DNA strand tethered on AuNPs, and the 5′ end triggered the HCR to produce a long DNA polymer. Thus, a small amount of target

* Corresponding authors. Fax: +86 84022680.
E-mail addresses: adani@engr.ucr.edu (A. Mulchandani), sc169@163.com (C. Shi).

[1] *Abbreviations used:* AuNPs, gold nanoparticles; HCR, hybridization chain reaction.

http://dx.doi.org/10.1016/j.ab.2014.04.022
0003-2697/

DNA strands could provide substantial negative charges on AuNPs, preventing the individual red AuNPs from forming blue aggregates at relatively high salt concentrations. Using this enzyme-free and signal amplification strategy, we were able to detect target DNA at concentrations as low as 0.5 nM with the naked eye.

Materials and methods

Reagents and materials

All oligonucleotides (HPLC purified) used in this work (Table S1) were supplied by SBS Genetech. Co. Ltd.

Gold nanoparticles with an average diameter of 15 nm were synthesized using the citrate reduction protocol reported previously [31]. The concentration of AuNPs (~2.8 nM) was calculated by comparing the number of Au atoms per particle to the total number of Au atoms in the solution [32]. AuNPs were functionalized with thiol-modified oligonucleotides according to the method described in the literature [33]. The functionalized AuNPs were purified by centrifugation and removal of supernatant. Then the final particles were resuspended in sodium phosphate buffer (10 mM, 0.3 M NaCl, pH 7.5).

Gel electrophoresis of HCR products

The HCR system was verified by using agarose gel electrophoresis. To ensure that H1 and H2 formed hairpin monomers, we annealed the hairpin strands by heating for 3 min at 95 °C in sodium phosphate buffer, and then allowing them to cool to room temperature. H1 and H2 were mixed in different tubes, to which different concentrations of target DNA were added. The final concentration of each hairpin—H1 and H2—was 1 μM. Hybridization chain reactions were performed at room temperature for 4 h. These reaction samples were then run on a 2% agarose gel for 30 min at 100 V and imaged under UV light.

Unamplified colorimetric DNA detection

We investigated the sensitivity of the colorimetric sensor without HCR with the naked eye. The DNA-functionalized AuNPs were incubated with various concentrations of target DNA (total volume 10 μL) for 10 min at room temperature, followed by the addition of 0.5 μL of 1 M $MgCl_2$ for colorimetric detection. The color changes of solutions were observed by the naked eye, and photographed 1 min after the addition of $MgCl_2$ (Fig. S1).

Amplified detection of target DNA

Varied concentrations of target DNA were added to the mixture of AuNPs and hairpins H1 and H2 in different tubes. The final volume of every sample was 20 μL, and both the final concentration of H1 and that of H2 were 10^{-8} M in each sample. The mixtures were incubated at room temperature for 4 h, followed by the addition of 1 μL of 1 M $MgCl_2$. The color changes of solutions were observed by the naked eye and photographed. Finally, the samples were diluted to 1200 μL and the 400- to 750-nm absorption spectrum of each sample was recorded with a UV/Vis spectrophotometer in 20 min.

Results and discussion

Design of colorimetric sensor

Fig. 1 depicts the colorimetric DNA detection by enzyme-free signal amplification and gold nanoparticle. In this method, target DNA was divided into two regions (Table S1). The first region hybridized with a DNA-functionalized AuNP, and the second region as an initiator triggered a hybridization chain reaction. We used HCR to achieve signal amplification and improve sensitivity of

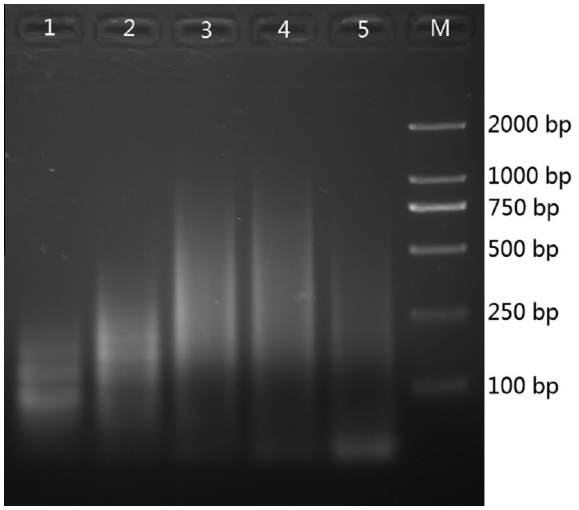

Fig.2. Gel electrophoresis of HCR products induced by different concentrations of target DNA containing 1 μM mixture of H1 and H2. Lane M, DL 2000 DNA Marker; Lanes 1–5, 3, 1, 0.3, 0.1, and 0 μM, respectively, of target DNA.

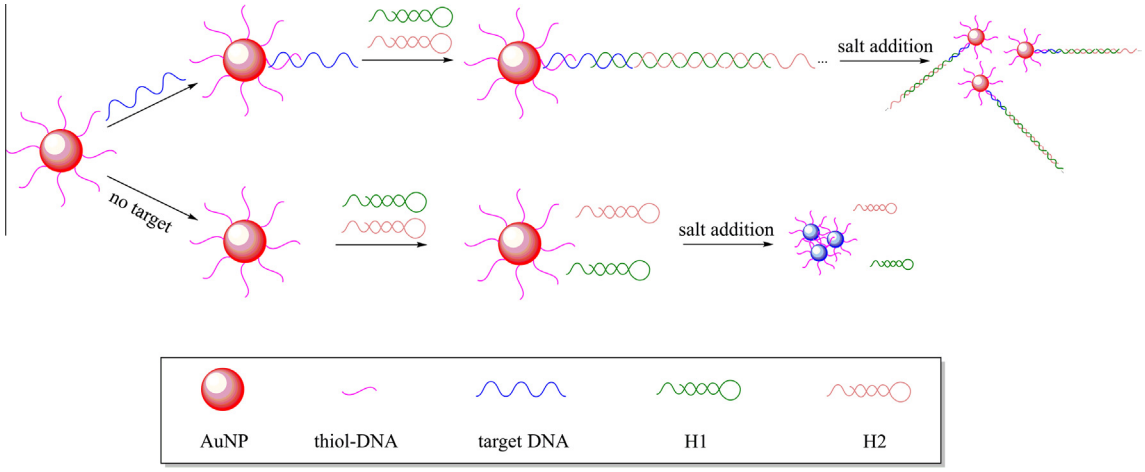

Fig.1. Schematic representation of colorimetric DNA detection by enzyme-free signal amplification and gold nanoparticle.