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Utilization of unmodified gold nanoparticles for label-free detection of mercury (II): Insight into rational design of mercury-specific oligonucleotides

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HIGHLIGHTS

- A rational design of Hg^{2+}-specific ssDNA (MSO) was proposed for the colorimetric biosensing system using unmodified AuNPs as indicators.
- The leftover bases of MSO as a result of T–T mismatch can absorb on the AuNPs.
- The DLS proved the size increase of MSO:DNA conjugate due to Hg^{2+} addition.
- An improved LOD value by ~20-fold was achieved by cutting the leftover bases.

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ABSTRACT

Colorimetric detection of mercury (II) with the use of DNA oligonucleotides and unmodified gold nanoparticles (AuNPs) as indicators has been extensively studied. This study provides in-depth insights into the rational design of mercury-specific oligonucleotides (MSO) in the biosensing system. The leftover bases of MSO, as a result of the formation of T–Hg^{2+}–T base pairs, can adsorb on the AuNPs and hinder their aggregation at concentrations of salt. This phenomenon was directly verified by the changes in particle sizes characterized by dynamic light scattering for the first time. Based on these findings, we proposed a rational design for the MSO with approximately 20-fold improvement in detection sensitivity. The detection limit of the proposed assay decreased to 15 nM with a linear working range from 50 nM to 300 nM for Hg^{2+}. The cross-reactivity against eight other metal ions was negligible compared with the response to Hg^{2+}. Considering the diverse applications of AuNPs with oligonucleotides, this study can serve as a good reference and provides important implications in sensing and DNA-directed nanoparticle assembly.

1. Introduction

Gold nanoparticles (AuNPs) exhibit a strong dependent absorbance feature referred to as local surface plasmon resonance (LSPR) [1–3]. AuNPs can be aggregated to induce the red shift of the SPR depending on the aggregation degree [1,4,5], whereas, the tunable optical property provides a quantitative basis for the label-free colorimetric detection of metal ions, such as Hg^{2+} [5,6], Pb^{2+} [7,8], and Cd^{2+} [9], as well as proteins and small molecules [10,11]. Among these recognized biosensing elements, oligonucleotide-based colorimetric bioassay using AuNPs as an indicator has received increased attention in the past decade because of its simplicity, speed, ease of use, and colorimetric visualization [7,8,12]. Compared to established approaches, the use of unmodified AuNPs as the colorimetric probe can mitigate a number of concerns, such as complex surface modifications, hard labeling, regeneration issues, and unstable real samples. Thus, this approach attracted considerable attention as a simple, label-free, and rapid visual detection assay with a huge potential for field analysis [5,6,11,13–15]. Moreover, the label-free detection strategy based on the DNAzyme-AuNPs system designed for UO_2^{2+} detec-

Abbreviations: AuNPs, gold nanoparticles; DLS, dynamic light scattering; HR-TEM, high resolution transmission electron microscopy; LSPR, local surface plasmon resonance; MBs, magnetic beads; MOPS, 3-morpholinopropane-1-sulfonic acid; MSO, mercury-specific oligonucleotides; PDI, poly disparity index; RT, room temperature; TEM, transmission electron microscopy; T–Hg^{2+}–T, thiamine-mercury-thiamine.

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tion indicated a higher sensitivity compared with the labeled one [16], which confirms to the practical applications of unmodified AuNPs used as the colorimetric probe.

Since the first reports of the preferential advantage of ssDNA over dsDNA sequence while adsorbing on the surface of AuNPs [17], extensive studies on developing sensing strategies based on the AuNPs and DNA oligonucleotides for the colorimetric and label-free detection of contaminants in aqueous media have been made. Owing to its high toxicity, persistence, and bioaccumulative essence mercury ion (Hg^{2+}) is of great concern in global water environment safety. In 2004, Ono and Togashi first reported that mercury ions can bind in between two T-bases and form the stable T–Hg^{2+}–T base pairs [18]. Numerous literature has demonstrated the sensing detection system of Hg^{2+} by taking advantages of the oligonucleotides interacting differently in single-/double-stranded states (ssDNA/dsDNA) on the AuNPs. Which is mainly based on the sequence length, concentration, electrostatic properties, ionic interaction, hybridization, and redistribution of charge, while adsorbing AuNPs [4,10,19,20]. However, in-depth insights into the interaction of the unmodified AuNPs to oligonucleotide are still rare because of the lack of suitable characterization techniques. This lack hinders our understanding on the conformation, complete hybridization, and aggregation of oligonucleotide sequences with the unmodified AuNP-based bioassays.

The considerable interest to learn more about the conformation nature of DNA on AuNP surface is evident. Recently, the conformation of DNA tethered onto the surface of AuNPs via the thiol-Au bond has been reported using techniques such as gel electrophoresis [21,22]. The electrophoretic isolation and conformation of AuNPs to DNA conjugates include limitations in isolating discrete nanocrystal/DNA conjugates when the size of oligonucleotide falls below 50 bases [22] even though shorter DNA has more practical applications than longer ones. Furthermore, it was argued that the soft DNA shell around the rigid Au cores be squeezed or compressed more while migrating through the gel with higher agarose concentration [23]. Moreover, AuNPs aggregates while being applied with the electric charge in the system, hence, AuNPs may require pre-coating with stabilizing chemicals; however, the pretreatment of AuNPs with chemicals may distort the DNA conformation [20]. Dynamic light scattering (DLS) is recently demonstrated as a sensitive probe to characterize the tethered DNA conformation on AuNPs because of its prominent label-free and complete non-invasion advantages [20,24,25]. These results will also shed light on the characterization of DNA conformation to the surface of unmodified AuNPs and inspire us to design experiments accordingly.

As a consequence, this study aims to develop a label-free Hg^{2+} detection by utilizing unmodified AuNPs as indicators through which we can gain insights on the rational design of mercury-specific oligonucleotides. The DLS was chosen as a tool to characterize DNA conformation to unmodified AuNPs. The outcomes of this study will offer in-depth insights into our understanding of the target-induced DNA conformation change to the unmodified AuNPs and guide us in rationally designing the DNA-based biosensing strategy with unmodified AuNPs as the colorimetric probe. Considering the diverse applications of AuNPs with oligonucleotides, this study can serve as a good reference in providing important implications in the sensing and DNA-directed nanoparticle assembly.

2. Experiment

2.1. Chemicals and apparatuses

Two mercury-specific oligonucleotides (MSO) sequences were chosen to investigate the rational design of the DNA using unmodified AuNPs as the colorimetric probe. The MSO sequence reported by Prof. Lu's group (Seq-1: 5′-TCA TGT TTG TTT GTT GGC CCC CCT TCT TTC TTA-3′), [26] was selected for the investigation. Another sequence was designed from the originally reported sequence by deleting five nucleotide base pairs on the 5′-end (Seq-2: 5′-TTT GTT TGT TGG CCC CCC TTC TTT CTT A-3′). All sequences were synthetically produced by Takara Biomed (Beijing, China). Chloroauric acid ($HAuCl_4$) was obtained from Sinopharm Chemical Reagent Company (Shanghai, China). Tri-Sodium Citrate ($C_6H_5Na_3O_7{\cdot}2H_2O$) and all metallic ions of standard analytical grade were bought from Beijing Chemical Reagent Company (Beijing, China). 3-morpholinopropane-1-sulfonic acid ($C_7H_{15}NO_4S$) MOPS and Sodium Nitrate ($NaNO_3$) were purchased from Amresco (Ohio, USA). The magnetic separation stand (twelve-position) was from Promega (Madision, WI, USA). Metal ions were used and diluted with Milli-Q water of 18 Ω cm obtained from a Millipore system. All solutions were prepared using molecular biology grade USP sterile purified water (RNase-, DNase- and protease- free).

Absorption spectra were recorded using a U-3900 UV–vis spectrophotometer (Hitachi, Japan) with a 1 cm Quartz sample cell. Transmission electron microscopy (TEM) sample and high resolution transmission electron microscopy (HR-TEM) measurements were made at an accelerating voltage of 80 kV and 200 kV on the TEM system H7650B (Hitachi, Japan) and the JEM 2100 (JEOL, Japan), respectively. All samples for characterization on TEM were prepared by placing a drop of the sample solution on a carbon-coated copper grid, which was kept to dry at room temperature (RT) before analysis. Kinetic analysis was conducted using fluorescence spectrophotometer F-7000 (Hitachi, Japan) and DLS measurements were analyzed with Zetasizer Nano ZS90 (Malvern Instruments Ltd., England) at RT.

2.2. Synthesis of AuNPs

AuNPs were synthesized using the citrate reduction method [6,14,19,27]. A 10 mL solution of freshly prepared sodium citrate (38.8 mM) was briefly and rapidly injected into a 100 mL boiling solution of $HAuCl_4$ (1 mM), which was vigorously stirred continuously in an oil bath for another 30 min. The color of the solution changed to red-wine which was cooled to RT, and then, filtered with a 0.45 μm filtration assembly to remove the precipitate. The filtered colloidal solution was stored at 4 °C for further use. Synthesized AuNPs were compared with 13 nm-sized commercial NPs. The concentration of synthesized AuNPs was estimated through the Lambert-Beer Law by using the extinction coefficient of $2.01 \times 10^8\ M^{-1}\ cm^{-1}$ at a 520 nm wavelength [28,29]. The size and monodispersity of the AuNPs were further confirmed by HR-TEM and DLS.

2.3. Kinetic analysis between the MSO sequence and Hg^{2+}

The reaction between the functional nucleic acids and its target has been studied using the evanescent wave all-fiber biosensing platform [28] and the surface plasmon resonance technology [30]. To investigate the binding ability of the two selected MSO sequences specific for Hg^{2+}, a simple binding assay that analyzes binding affinity and a dissociation constant (K_d) were conducted following the protocol described by Zhou's group [9]. Seqs-1 and -2 were synthesized by labeling with FAM on the 5′-end. The complementary strands of ssDNA (c-DNA) with varying lengths from 10 to 12 bp (K_d—C_{10}: 5′-CCC CCC CCC CCC TAA GAA AGA A-3′; K_d-C_{11}: 5′-CCC CCC CCC CCC TAA GAA AGA AG-3′; K_d-C_{12}: 5′-CCC CCC CCC CCC TAA GAA AGA AGG-3′) were labeled with biotin on the 5′-end and bound with streptavidin modified magnetic beads (MBs). Afterward, the MSO sequences were immobilized onto the MBs through hybridization with the c-DNA. The amounts of immobilized MSOs