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Colorimetric detection of mercury ion (Hg^{2+}) based on DNA oligonucleotides and unmodified gold nanoparticles sensing system with a tunable detection range

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ABSTRACT

Here, we report a simple and sensitive colorimetric detection method for Hg^{2+} ions with a tunable detection range based on DNA oligonucleotides and unmodified gold nanoparticles (DNA/AuNPs) sensing system. Complementary DNA strands with T–T mismatches could effectively protect AuNPs from salt-induced aggregation. While in the presence of Hg^{2+} ions T–Hg^{2+}–T coordination chemistry leads to the formation of DNA duplexes, and AuNPs are less well protected thus aggregate at the same salt concentration, accompanying by color change from red to blue. By rationally varying the number of T–T mismatches in DNA oligonucleotides, the detection range could be tuned. Employing duplex oligonucleotides with 4 T–T mismatches in the sensing system, a sensitive linear range for Hg^{2+} ions from 0 to 5 μM and a detection limit of 0.5 μM are obtained. Adding the number of T–T mismatches to 6 and 8, the assay region is enlarged and linear range is tuned. A low proportion of T–T mismatches makes the detection range narrow but the sensitivity high while a high proportion influences the detection limit but enlarges assay region. Besides, the sensor also shows a good selectivity for Hg^{2+}.

1. Introduction

Colorimetric sensors based on DNA oligonucleotides and gold nanoparticles (DNA/AuNPs) attract much attention in recent years for the convenience of visual observation and easy operation. In such sensing systems, DNA strands serve as recognition units for not only their complementary oligonucleotides but a variety of targets known as DNAzymes and aptamers. Gold nanoparticles serve as sensing elements for their unique optical properties dependent on inter-particle distance. Incorporating oligonucleotides and gold nanoparticles, colorimetric sensors for oligonucleotides (Storhoff et al., 1998; Sato et al., 2003; Li and Rothberg, 2004a,b), metal ions such as Pb^{2+} (Liu and Lu, 2003; Wei et al., 2008; Z.D. Wang et al., 2008), K^+ (Wang et al., 2006), UO_2^{2+} (Lee et al., 2008), small molecules such as adenosine (Liu and Lu, 2004), cocaine (Liu and Lu, 2006; Zhang et al., 2008), ATP (Wang et al., 2007; Chen et al., 2008), proteins such as thrombin (Wei et al., 2007) have been successfully developed. Among these, colorimetric assays for metal ions are given more concern in practical use for the contamination of environment caused by heavy metal ions.

Mercury ion (Hg^{2+}) is a highly toxic pollutant to human beings (Sekowski et al., 1997). Since the T–Hg^{2+}–T coordination was proposed (Miyake et al., 2006; Tanaka et al., 2007), a variety of colorimetric sensors based on DNA/AuNPs for Hg^{2+} have been developed. Mirkin's group modified single thymine mismatched oligonucleotides onto AuNPs and achieved Hg^{2+} assay through the melting temperature change of aggregates (Lee et al., 2007). Liu's group improved that method through optimizing the sequences and lowering the melting temperature of DNA–AuNPs conjugates thus made operation simply done at ambient temperature (Xue et al., 2008). Willner' group developed unmodified colorimetric sensors based on the fact that random-coil ssDNA could effectively protect AuNPs from salt-induced aggregation while Hg^{2+}-mediated hairpin structure of DNA could not (Li et al., 2008). Chang's group designed the sensor employing poly-T ssDNA and the folded structure of Hg^{2+}–DNA complex induced the aggregation of AuNPs in the presence of salt (Liu et al., 2008). Recently, other colorimetric sensors for Hg^{2+} such as the rapid and portable detection device (He et al., 2008), colorimetric and turn-on fluorescent probes (H. Wang et al., 2008) have been reported.

Mercury is widely distributed in soil, lakes or rivers and exists with different concentrations (Miller et al., 1996). Mercury vapor evaporates from earth surface and is converted to mercury ion then returns to earth in rainwater (Clarkson et al., 2003). Besides the fact

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that samples from different locations contain different concentration of Hg^{2+}, the detection criterion for different cases may also vary. Though a high sensitivity has been achieved, colorimetric sensors using DNA/AuNPs for Hg^{2+} developed usually obtain one detection range and the range is relatively narrow (typically from 0 up to about 10 μM). Some sensors reach the detecting saturation when treated with high concentration of Hg^{2+} (Xue et al., 2008; Liu et al., 2008). Thereby besides the sensitivity, the tunable detection range is also an important issue. Colorimetric sensors for another polluting metal ion, Pb^{2+}, have been developed with tunable dynamic range by adjusting the ratio between active and inactive DNAzymes (Liu and Lu, 2003) or by regulating pH value (Z.D. Wang et al., 2008). However, to our knowledge, colorimetric methods in Hg^{2+} detection focusing on a tunable detection range have not been reported.

In this paper, a simple and sensitive colorimetric detection method for Hg^{2+} with a tunable detection range based on DNA oligonucleotides and unmodified gold nanoparticles sensing system is developed. Oligonucleotides with T–T mismatches retain random-coil in the absence of Hg^{2+} ions. These DNA units could effectively protect AuNPs from salt-induced aggregation (Li and Rothberg, 2004a,b). While in the presence of Hg^{2+}, DNA oligonucleotides could form duplex structures due to T–Hg^{2+}–T coordination chemistry (Miyake et al., 2006). The rigid structures could not stabilize individual red AuNPs after salt was added, leading to a color change to purple or blue. The assay region, linear range, detection limit as well as the color displayed could be tuned by varying the number of T–T mismatches in DNA duplexes. The visual observation together with a tunable detection range provide a convenient way of estimating Hg^{2+} in the test sample and may fit different detecting needs.

2. Experimental

2.1. Chemicals and materials

Chloroauric acid ($HAuCl_4$) was obtained from Shanghai Chemical Reagent Company (Shanghai, China). Sodium citrate and sodium perchlorate ($NaClO_4$) were bought from Beijing Chemical Reagent Company (Beijing, China). Mercury perchlorate hydrate ($Hg(ClO_4)_2$) was purchased from Aldrich. 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) was obtained from Sigma. Other chemicals involving metallic salts were of analytical grade and were used as received.

All DNA oligonucleotides were synthesized in Sangon Biotechnology Inc. (Shanghai, China). Their base sequences were designed as follows: DNA1 (5′-ATG**T**CACG**T**TAT**T**GCA**T**TCG-3′) and its 4-mer T–T mismatched bases oligonucleotide DNA2 (5′-CGA**T**TGC**T**ATA-**T**CGTG**T**CAT-3′), DNA3 (5′-AT**T**GC**T**CG**T**TA**T**GC**T**AT**T**CG-3′) and its 6-mer T–T mismatched bases oligonucleotide DNA4 (5′-CG**T**AT**T**GC**T**-TA**T**CG**T**GC**T**AT-3′), DNA5 (5′-AT**T**GC**TT**G**T**TA**TT**C**T**AT**T**CG-3′) and its 8-mer T–T mismatched bases oligonucleotide DNA6 (5′-CG**T**AT**T**G**TT**TA**T**C**TT**GC**T**AT-3′), where the bold T represents the mismatched thymine base. DNA work solution was obtained by dissolving oligonucleotides in 10 mM HEPES buffer containing 100 mM $NaClO_4$ (pH 7.4) and was stored at 4 °C before use. The concentration of oligonucleotides was determined using the 260 nm UV absorbance and the extinction coefficients were calculated by the sum of the extinction coefficients of the individual bases: $\varepsilon(dA) = 15{,}400\ M^{-1}\ cm^{-1}$, $\varepsilon(dG) = 11{,}500\ M^{-1}\ cm^{-1}$, $\varepsilon(dC) = 7400\ M^{-1}\ cm^{-1}$, and $\varepsilon(dT) = 8700\ M^{-1}\ cm^{-1}$. *Note*: As reported previously by Lu's group (Z.D. Wang et al., 2008; Lee et al., 2008), the process of checking the exact concentration of DNA strands is very important for small amount of unhybridized ssDNA could also stabilize AuNPs and thus interfere the sensitivity.

2.2. Instrumentation

Absorption spectra were recorded on a Cary 50 Scan UV-Visible spectrophotometer (Varian, USA) at room temperature. Transmission electron microscopy (TEM) measurements were made on a Hitachi H-8100 transmission electron microscope operated at an accelerating voltage of 200 kV. The samples for TEM characterization were prepared by adding a drop of colloidal solution on a carbon-coated copper grid and drying at room temperature.

2.3. Synthesis of AuNPs

Gold nanoparticles (13 nm diameter) were synthesized by reduction of $HAuCl_4$ by sodium citrate (Grabar et al., 1995). Briefly, a solution of sodium citrate (10 mL, 38.8 mM) was rapidly added to a vigorously stirred boiling aqueous solution of $HAuCl_4$ (100 mL, 1 mM). After a continuous boiling for 10 min, the mixed solution was stirred for additional 15 min. Then the solution was cooled to room temperature and filtered, stored in a refrigerator at 4 °C before use. The concentration of AuNPs was estimated to be 13.4 nM, which was calculated from the quantity of the starting material ($HAuCl_4$) and the size of nanoparticles.

2.4. Colorimetric detection of Hg^{2+} ions

For the detection of Hg^{2+} ions, 10 μL of 1.8 μM DNA1 work solution was mixed with 10 μL of 1.8 μM DNA2 work solution, and then 40 μL of different concentration of Hg^{2+} solution (the test solution) was added. After a gentle vortex for 1 min, the mixture was allowed to react for 30 min. Subsequently 100 μL AuNPs was mixed with the reaction mixture and the solution was kept for 1.5 min, and then 100 μL of 0.24 M $NaClO_4$ was added, followed by either visual observation or UV–vis characterization. The assays were performed in 0.5 mL microcentrifuge tubes at room temperature (21–22 °C). *Note*: The room temperature should not be too low (>20 °C), or it may cause detecting disturbance in the designed sensing system. (Detailed reason was explained in the supplementary material.)

For the detection of Hg^{2+} ions using other DNA oligonucleotides, assay protocols were done as mentioned above except for that DNA3 work solution was mixed with DNA4 work solution, DNA5 work solution was mixed with DNA6 work solution, respectively.

For the detection of other metal ions, assay protocols were done as the same as Hg^{2+} detection. All the concentrations of the metal ions referred in the article were the original concentrations of the 40 μL test solution.

3. Results and discussion

3.1. The designed DNA1/DNA2/AuNPs sensing system for Hg^{2+} detection

Fig. 1A shows UV–vis absorption spectra and the corresponding photographs of the designed DNA1/DNA2/AuNPs sensing system treated with water or 5 μM Hg^{2+} test solution. As shown, in the absence of Hg^{2+} ions, a characteristic surface plasmon resonance absorption band of AuNPs was observed in the spectra at approximate 520 nm (Fig. 1A, curve a) and the color of the solution remained red after the addition of salt (final concentration of $NaClO_4$: 100 mM) (Fig. 1A, inset a). DNA1 and DNA2 retained their random-coil due to the four detached T–T mismatches, thus could adsorb on to the surface of AuNPs through the electrostatic attraction (Li and Rothberg, 2004a,b) and the interaction between the nitrogen-containing bases and gold (Wang et al., 2007; Zhang et al., 2008), which effectively protected AuNPs from salt-induced aggregation. Dispersed gold nanoparticles still existed after the addition