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DNAzyme-based colorimetric sensing of lead (Pb^{2+}) using unmodified gold nanoparticle probes

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Abstract

Novel functional oligonucleotides, especially DNAzymes with RNA-cleavage activity, have been intensively studied due to their potential applications in therapeutics and sensors. Taking advantage of the high specificity of 17E DNAzyme for Pb^{2+}, highly sensitive and selective fluorescent, electrochemical and colorimetric sensors have been developed for Pb^{2+}. In this work, we report a simple, sensitive and label-free 17E DNAzyme-based sensor for Pb^{2+} detection using unmodified gold nanoparticles (GNPs) based on the fact that unfolded single-stranded DNA could be adsorbed on the citrate protected GNPs while double-stranded DNA could not. By our method the substrate cleavage by the 17E DNAzyme in the presence of Pb^{2+} could be monitored by color change of GNPs, thereby Pb^{2+} detection was realized. The detection of Pb^{2+} could be realized within 20 min, with a detection limit of 500 nM. The selectivity of our sensor has been investigated by challenging the sensing system with other divalent metal ions. Since common steps such as modification and separation could be successfully avoided, the sensor developed here could provide a simple, cost-effective yet rapid and sensitive measurement tool for Pb^{2+} detection and may prove useful in the development of sensors for clinical toxicology and environmental monitoring in the future.

S Supplementary data are available from stacks.iop.org/Nano/19/095501

(Some figures in this article are in colour only in the electronic version)

1. Introduction

A large number of novel functional oligonucleotides such as aptamers and deoxyribozymes/ribozymes (DNAzymes/RNAzymes) have been selected *in vitro* [1, 2]. Aptamers are functional DNA or RNA structures that possess high recognition ability to specific molecular targets ranging from small inorganic or organic substances to even a protein or cell [3–11], while DNAzymes/RNAzymes [12–15] are catalytic nucleic acids capable of catalyzing a broad range of reactions including cleaving nucleic acid substrates [16–18], ligation [19], phosphorylation [20] and porphyrin metallation [21]. Among all these *in vitro* selected DNAzymes, particular attention has been paid to DNAzymes with RNA-cleavage activity due to their potential applications in therapeutics [22–24] and sensors [25–28].

The catalytic activity of some DNAzymes is divalent metal ion specific, just as the catalytic activity of some protein enzymes is metal ion cofactor dependent [13, 29, 30]. Taking advantage of this property, DNAzyme-based sensors have been designed for metal ion detection. Much research has been focused on the 17E DNAzyme due to its high specificity for Pb^{2+} and it shows great promise for specific metal ion Pb^{2+} sensors [13, 31].

Metal ion Pb^{2+} detection is of great importance to, for example, human health and environmental monitoring. In order to meet these objectives, a number of analysis methods have been developed over past decades, including classical atomic absorption/emission spectrometry [32, 33], electrochemical means [34], fluorescence techniques [35], mass spectra [36], photonic crystals [37], and so on. Recently, varieties of DNAzyme-based analytical methods have been developed for Pb^{2+} detection, including electrochemistry [38] and fluorescence [39–43].

Simple colorimetric sensors could potentially eliminate the use of analytical instruments and are attracting more and more attention [44–50]. A strategy for evolving colorimetric Pb^{2+} sensors has recently been developed by Lu and co-workers based on 17E DNAzyme, involving gold nanoparticles (GNPs) as sensing elements [44, 48–50]. Their colorimetric methods require steps such as modifying the DNAzyme (or its substrate) onto the GNPs and separating the modified GNPs from the unmodified or surplus DNAzyme (or its substrate). These steps usually lead to complication and a relatively high cost of the experiments. Developing modification-free GNP colorimetric sensors to simplify the detection process would be important and attractive. Rothberg's group reported a novel DNA sensor using colorimetric sensing with unmodified GNPs based on discriminated effects of different DNA structures on the nanoparticles [51, 52]. Recently, the targets of the unmodified methods have been extended to metal ions and proteins [53, 54].

In this work, we adopted a similar strategy to Lu's [48–50] to develop a simple, sensitive and label-free DNAzyme-based sensor for Pb^{2+} detection using unmodified GNPs [55] based on the fact that unfolded single-stranded (ss)DNA could be adsorbed on the citrate protected GNPs while double-stranded (ds)DNA could not. Thus unfolded ssDNA could stabilize the GNPs in the presence of a given high concentration of salt while dsDNA could not, and the GNPs aggregated in the presence of the same concentration of salt [51, 52]. By our method substrate cleavage by the 17E DNAzyme in the presence of Pb^{2+} could be monitored by the color change of GNPs, thereby Pb^{2+} detection was realized (figure 1). The detection of Pb^{2+} could be realized within 20 min, with a detection limit of 500 nM. The selectivity of our sensor has been investigated by challenging the sensing system with other divalent metal ions. Since common steps such as modification and separation could be successfully avoided, the sensor developed here could provide a simple, cost-effective yet rapid and sensitive measurement tool for Pb^{2+} detection and has the potential for application in future GNP colorimetric sensors.

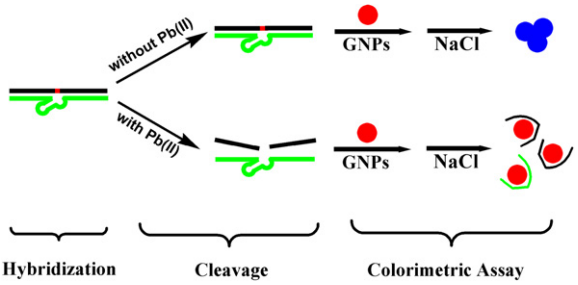

Figure 1. Strategy for Pb^{2+} detection using GNP colorimetry. 17E DNAzyme was first hybridized with its substrate to form the duplex. In the presence of Pb^{2+}, the substrate strand was cleaved, resulting in the release of fragments which could be adsorbed on the GNP probes and thus could stabilize the GNP probes in the presence of a given high concentration of salt (the red solution appears). In the absence of Pb^{2+}, however, the remaining duplex could not be adsorbed on the GNP probes and thus could not stabilize the GNP probes in the presence of a given high concentration of salt (the blue solution appears due to the aggregation of GNP probes induced by the salt).

2. Experimental section

2.1. Chemicals and materials

Chloroauric acid ($HAuCl_4$) was purchased from the Shanghai Chemical Reagent Company (Shanghai, China). Sodium citrate, sodium chloride, lead acetate, zinc acetate, cobalt chloride, magnesium chloride, manganese chloride, nickel chloride, cadmium chloride, calcium chloride, and copper sulfate were purchased from the Beijing Chemical Reagent Company (Beijing, China). Other reagents and chemicals were at least of analytical reagent grade. The water used throughout all experiments was purified by a Milli-Q system (Millipore, Bedford, MA, USA). 20 mM Tris-HCl buffer containing 140 mM NaCl, 5 mM KCl (pH 7.5) was used throughout the experiments.

All the oligonucleotides used were purchased from the TaKaRa Biotechnology Co., Ltd (Dalian, China). The sequence of 17E DNAzyme (abbreviated as 17E) was 5′ CAT CTC TTC TCC GAG CCG GTC GAA ATA GTG AGT 3′, the sequence of the cleavage substrate (abbreviated as 17DS) was 5′ ACT CAC TAT $\boldsymbol{rA}$ GGA AGA GAT G 3′ (note: the substrate was a DNA/RNA chimera in which $\boldsymbol{rA}$ represents the ribonucleotide adenosine) [13, 45–47]. The proposed secondary of the 17E/17DS duplex is shown in the supporting information (figure S1 (available at stacks.iop.org/Nano/19/095501)).

The concentrations of oligonucleotides were determined using the 260 nm UV absorbance and the extinction coefficients were calculated by the sum of the extinction coefficients of the individual bases: $\varepsilon(\mathrm{d}A) = 15\,400\ \mathrm{M}^{-1}\,\mathrm{cm}^{-1}$, $\varepsilon(\mathrm{d}G) = 11\,500\ \mathrm{M}^{-1}\,\mathrm{cm}^{-1}$, $\varepsilon(\mathrm{d}C) = 7400\ \mathrm{M}^{-1}\,\mathrm{cm}^{-1}$, $\varepsilon(\mathrm{d}T) = 8700\ \mathrm{M}^{-1}\,\mathrm{cm}^{-1}$.

2.2. Instrumentation

Absorption spectra were recorded on a Cary 500 Scan UV–vis–NIR spectrophotometer (Varian, Harbor City, CA, 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 placing a drop of colloidal solution on a carbon-coated copper grid and drying at room temperature.

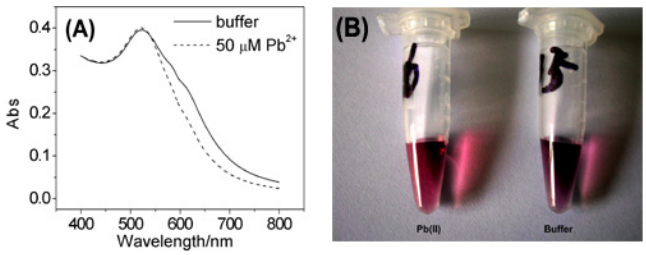

Figure 2. Colorimetric detection of Pb^{2+}. (A) Typical UV–visible absorption spectra and (B) photographs of 200 μl GNPs/17E–17DS duplex solutions in the absence (solid line) and in the presence (dashed line) of 50 μMPb^{2+} after addition of 20 μl 0.5 M NaCl.

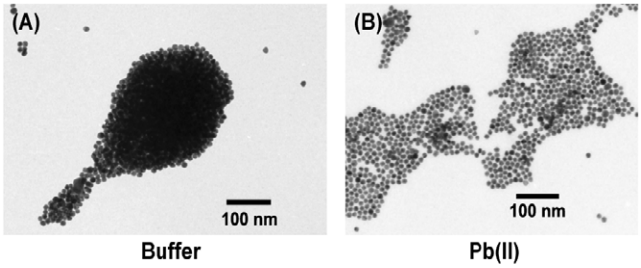

Figure 3. TEM images of the 13 nm GNPs mixed with 17E–17DS duplex solutions in the absence (A) and presence of 50 μM Pb^{2+} (B) after addition of 39 mM NaCl. (The scale bar represents 100 nm.)

2.3. Preparation of GNPs

13 nm GNPs were prepared according to a literature method [55]. Briefly, a sodium citrate solution (0.1 M, 1.94 ml) was rapidly added to a boiled $HAuCl_4$ solution (50 ml H_2O, 0.167 ml 10% $HAuCl_4$) under vigorous stirring. The mixed solution was boiled for 10 min and further stirred for 15 min. The resulting wine-red solution was cooled to room temperature and filtered, then stored in a refrigerator at 4 °C before use.

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

A typical GNP colorimetric analysis was realized by the following procedure (see figure 1):

(I) The formation of the 17E–17DS duplex (see figure S1 (available at stacks.iop.org/Nano/19/095501)). 1 μM 17E–17DS duplex in Tris-HCl buffer was prepared by hybridizing 17E DNAzyme and its substrate 17DS with a molar ratio of 1:1 at 90 °C for 5 min, then slowly cooling to room temperature and storing at 4 °C before use.

(II) The cleavage reaction. 30 μl of the above-mentioned 1 μM 17E–17DS duplex was mixed with 15 μl Pb^{2+} solution of an appropriate concentration (or other divalent metal ions as control) in Tris-HCl buffer and held at 4 °C for 10 min.

(III) Colorimetric detection using GNPs. 200 μl 13 nm GNPs was mixed with 40 μl reaction mixture of 17E–17DS duplex and Pb^{2+} in step (II). The solutions were allowed to react for 5 min at room temperature and then 20 μl 0.5 M NaCl was added to produce a color change. Time-dependent UV–visible spectra of the mixing GNP/17E–17DS duplex/Pb^{2+} solution in the presence of NaCl were then immediately measured with a Cary 500 Scan UV–vis–NIR spectrophotometer.

3. Results and discussion

3.1. Mechanistic basis for the sensing system

The as-prepared GNPs are stable due to the electrostatic repulsion of the negative capping agents (i.e. citrate) against the van der Waals attraction between GNPs [55]. Therefore, addition of enough salt would screen the repulsion between the unmodified negatively charged GNPs and lead to the aggregation of the GNPs followed by a corresponding color change (from red to blue). As Rothberg *et al* reported previously, unfolded ssDNA could be adsorbed on the citrate-protected GNPs while dsDNA could not [51, 52]. Thus unfolded ssDNA could stabilize the GNPs in the presence of a given high concentration of salt, while dsDNA could not and the GNPs aggregated in the presence of the same concentration of salt. We just made use of this property to design our sensor. As shown in figure 1, in the presence of Pb^{2+} the 17E DNAzyme could cleave the substrate 17DS, which could release the ssDNA (including the 17E and the fragments from the 17DS) from the hybridized 17E–17DS duplex. The ssDNA could be adsorbed on the GNPs thus protecting the GNPs from aggregation in the presence of a given high concentration of salt (the solution appeared red). However, the 17E–17DS duplex could remain in the absence of Pb^{2+}, which could not adsorb on the GNP probes and thus could not stabilize the GNP probes under the same conditions (the solution appeared blue). Therefore, Pb^{2+} detection could be easily realized by monitoring the color change of the GNPs.

3.2. Colorimetric detection of Pb^{2+}

Figure 2 shows a typical colorimetric detection of Pb^{2+} using unmodified 13 nm GNP probes. In the presence of 50 μM Pb^{2+} the 17E DNAzyme could cleave its substrate 17DS, thus releasing the ssDNA fragments from the 17E–17DS duplex. These fragments could be adsorbed on the GNPs, which could protect the GNPs from aggregation induced by the added salt. Therefore a characteristic surface plasmon resonance (SPR) absorption band of the GNPs at about 530 nm was also observed (figure 2(A)) and the corresponding red color of the GNP solution appeared (figure 2(B)). As for the trial solution without Pb^{2+}, a SPR band at about 530 nm with a shoulder band at about 620 nm was observed (figure 2(A)) and the corresponding color of the GNP solution changed from red to purple as soon as 20 μL 0.5 M NaCl was added (figure 2(B)). Through this color change phenomenon, the cleavage of the substrate 17DS with the 17E DNAzyme could be directly monitored with the naked eye, realizing the detection of Pb^{2+} in a very convenient way.

Note that in the absence of Pb^{2+}, the GNPs aggregated together after addition of NaCl (figure 3(A)); while in the presence of 50 μM Pb^{2+}, the GNPs were not aggregated (figure 3(B)). This result was consistent with the red-shift of the

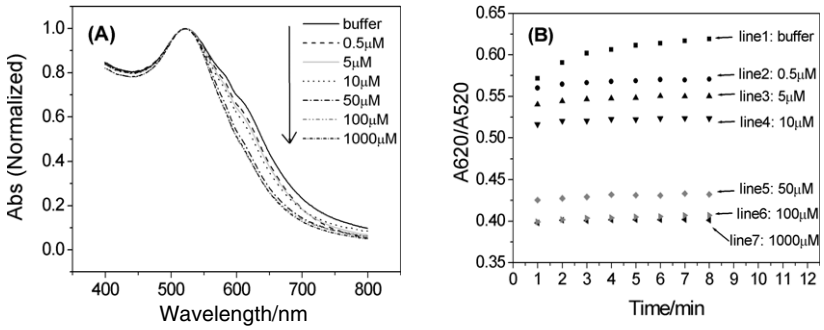

Figure 4. UV–visible absorption spectra (A) and plots of the time-dependent absorption ratio (A620/A520) over 8 min (B) of 200 μl GNP/17E–17DS duplex solutions in the presence of varying concentration of Pb^{2+} after the addition of 20 μl 0.5 M NaCl.

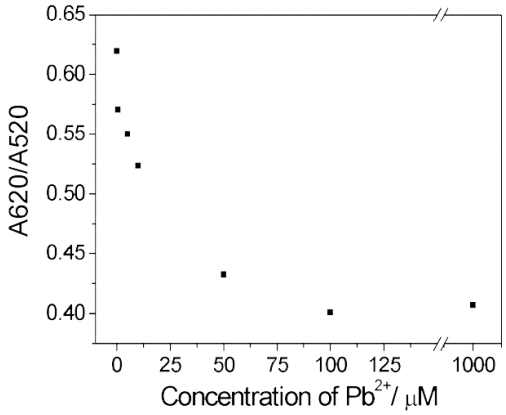

Figure 5. Plots of the absorption ratio (A620/A520) at 8 min versus Pb^{2+} concentration.

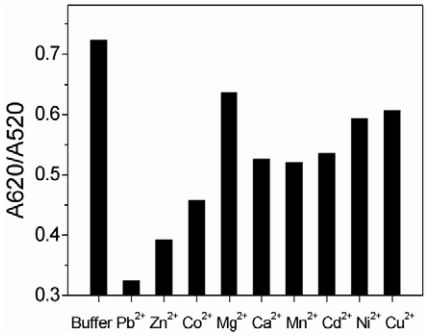

Figure 6. The absorption ratio (A620/A520) of 200 μl GNP/17E–17DS duplex solutions in the presence of 50 μM of different divalent metal ions after the addition of 20 μl 0.5 M NaCl.

UV–visible absorption spectra and the color change from red to purple of the GNP solution mixed with 17E–17DS duplex in the presence and absence of Pb^{2+} after addition of NaCl (see figure 2).

To quantitatively detect Pb^{2+} using our GNP colorimetric sensor, UV–visible spectra of 200 μl GNP solution mixed with 17E–17DS duplex in the absence and in the presence of different concentrations of Pb^{2+} after addition of 20 μl 0.5 M NaCl were recorded. The UV–visible data presented in figure 4 showed that the more Pb^{2+} that was provided for the 17E–17DS duplex cleavage reaction, the higher the catalytic activity of the 17E DNAzyme that was obtained, thus the more ssDNA could be released (figures 1 and 4 (see also figure S1 available at stacks.iop.org/Nano/19/095501)). These released ssDNA could then be adsorbed on the GNPs and protect the GNPs from aggregation after the addition of salt. With increasing Pb^{2+} concentration, a less obvious aggregation of the GNPs was observed (figure 4 and figure S2 (available at stacks.iop.org/Nano/19/095501)). As shown in figure 5, the color change of the GNPs was a sensitive function of the Pb^{2+} concentration. Significantly, as little as 500 nM Pb^{2+} could be detected using our sensing system. Note that the current 500 nM detection limit is not sufficient for environmental studies where lead must be detected at the 10–100 nM range. It should be pointed out that though the absorption ratio (A620/A520) decreased gradually with the increase of Pb^{2+} concentration, a linear response of absorption ratio versus Pb^{2+} concentration could not be obtained (figure 5).

3.3. Selectivity of the sensing system

To test whether the detection of Pb^{2+} is specific, control experiments were done using eight other divalent metal ions (Zn^{2+}, Co^{2+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Cd^{2+}, Ni^{2+}, and Cu^{2+}) (figure 6). These metal ions were tested because they showed relatively high catalytic activity toward the cleavage by 17E DNAzyme [40, 41, 56]. The same concentration (50 μM) of divalent metal ions was investigated, and only in the presence of Pb^{2+} did the GNP solution remain red; in the presence of other divalent metal ions the GNP solutions changed from red to blue after the addition of salt, indicating that Pb^{2+} had the highest catalytic activity. As quantitative data showed in figure 6, Zn^{2+} and Co^{2+} also showed a slight response to the sensor, which was in agreement with previous work [40, 41, 56]. Thus the sensor developed here showed relatively good selectivity.

4. Conclusions

In summary, a simple, cost-effective yet rapid and sensitive colorimetric sensor for Pb^{2+} detection using unmodified GNPs has been developed in this work. The cleavage of the substrate

by the 17E DNAzyme in the presence of Pb^{2+} could be monitored by color change of the unmodified GNP probes thereby realizing Pb^{2+} detection. The sensor reported here showed relatively good selectivity for Pb^{2+} over other divalent metal ions with a detection limit of 500 nM. Since the common modification and separation steps usually used for colorimetric assay could be successfully avoided, the sensing system demonstrated here shows the potential for application of GNP colorimetric sensors in the future.

Acknowledgments

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