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Short communication

Colorimetric biosensing of mercury(II) ion using unmodified gold nanoparticle probes and thrombin-binding aptamer

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ABSTRACT

A colorimetric assay for the determination of mercury(II) (Hg^{2+}) in the presence of lead(II) (Pb^{2+}) was demonstrated with unmodified gold nanoparticles (AuNPs) as probes and 15-mer thrombin-binding aptamer (TBA, 5'-GGTTGGTGTGGTTGG-3') as sensing elements. Upon the addition of Hg^{2+} or Pb^{2+}, TBA consisting of six thymidine units and nine guanosine units interacted specifically with both ions to form a hairpin-like or a quadruplex structure, respectively. As a result, these conformation changes facilitated the salt-induced AuNP aggregation. Subsequently, to eliminate Pb^{2+} interference in the determination of Hg^{2+}, a novel technique by the use of a characteristic wavelength of aggregated AuNPs instead of the universal masking agent of Pb^{2+} (2,6-pyridinedicarboxylic acid, PDCA) was herein proposed. A comparison of the absorption spectra of the aggregated AuNPs in the presence of Hg^{2+} and Pb^{2+} showed that the characteristic wavelength of the aggregated AuNPs (800 nm) facilitated the determination of Hg^{2+} in the presence of Pb^{2+}. The calibration curve showed that the absorbance value at 800 nm increased linearly over the Hg^{2+} concentration range of 0.39–8.89 μM with a limit of detection of 200 nM. Then, the assay was successfully employed to determine Hg^{2+} in several water samples.

1. Introduction

The monitoring of toxic metal ions in aquatic ecosystems is an important issue because these contaminants can have severe effects on human health and the environment (Campbell et al., 2003). Mercury ions (Hg^{2+}), the most stable form of inorganic mercury, damage the brain, nervous system, kidneys, and endocrine system (Clarkson et al., 2003; Zalups, 2000). Therefore, the detection of Hg^{2+} level in both environmental and biological samples is clearly important. To date, a plethora of methodologies, including atomic absorption/emission spectrometry (Dagnall et al., 1973; Suddendorf et al., 1981), electrochemistry (Han et al., 2009; Liu et al., 2009b), fluorimetry (Lee et al., 2009; Liu et al., 2009a) and so on have been reported. However, most of them require expensive and sophisticated instrumentation and/or involve time-consuming pretreatment steps prior to the analysis. Therefore, new analytical methods from alternative techniques are always useful, especially if the methods are simple, cost-effective and comparatively fast.

Colorimetry is generally well known, and commonly used for routine analysis. Many recent reports describe the application of chromophoric colorimetry for sensing of Hg^{2+} (Hashemi-Moghaddam et al., 2009; Perez-Hernandez et al., 2009; Wan et al., 2009). Nevertheless, they are either limited with respect to complexity, sensitivity, selectivity, kinetically unstable, or incompatible with aqueous environments.

To alleviate the difficulties that conventional chromogenic sensors encounter, some colorimetric methods based on unmodified gold nanoparticles (AuNPs) as reporting probes and thymine (T)-rich deoxyribonucleotides (DNAs) as recognition elements have been developed recently (Li et al., 2008, 2009; Liu et al., 2008; Wang et al., 2008; Xu et al., 2009; Yu et al., 2009). AuNPs are excellent reporting probes mainly due to their high extinction coefficients (3–5 orders of magnitude higher than those of organic dyes molecules) (Rosi and Mirkin, 2005) and distance-dependent optical properties (Mirkin et al., 1996). On the other hand, thymine-containing DNAs have specific molecular recognition functions towards Hg^{2+} (Miyake et al., 2006; Tanaka et al., 2007). Very recently, Miyake et al. (2006) and Tanaka et al. (2007) reported the stabilization of DNA duplexes via the formation of Hg^{2+} mediated base pair, thymine–Hg^{2+}–thymine (T–Hg^{2+}–T), and revealed that the T–Hg^{2+}–T coordination was more stable than the Watson–Crick A–T pair. Despite the high selectivity of thymine-containing oligonucleotides towards Hg^{2+}, some of these assays were often susceptible to severe interference from Pb^{2+} (Li et al., 2008; Liu et al., 2008; Xu et al., 2009; Yu et al., 2009). Thus, to eliminate interference from Pb^{2+} in the analysis of Hg^{2+} is a very important issue. At present, almost all assays employ the univer-

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sal masking agent of Pb^{2+} (2,6-pyridinedicarboxylic acid, PDCA) (Li et al., 2008; Liu et al., 2008; Norkus et al., 2003; Yu et al., 2009). However, the use of the masking agent generally increases analytical costs and experimental steps. Thus, a promising alternative technique is required to circumvent these drawbacks.

In this paper, a novel and simple colorimetric Hg^{2+} assay by measuring the increased absorbance resulted from salt-induced aggregation of unmodified citrate-coated AuNPs in the presence of Hg^{2+} and thrombin-binding aptamer (TBA) was presented. The interference from Pb^{2+} in colorimetric determination of Hg^{2+} could be easily removed by measuring the absorbance value at a characteristic wavelength of the aggregated AuNPs. The technique could avoid the use of the universal masking agent of Pb^{2+} (2,6-pyridinedicarboxylic acid, PDCA) and thus facilitated the sensitive and selective determination of Hg^{2+} in the presence of Pb^{2+}.

2. Materials and methods

2.1. Reagents and chemicals

Hydrogen tetrachloroaurate(III) tetrahydrate was obtained from Acros Organics (NJ, USA). Trisodium citrate and sodium chloride (NaCl) were purchased from Beijing Chemical Reagent Company (Beijing, China). Mercury(II) perchlorate hydrate was purchased from Sigma–Aldrich (Milwaukee, WI, USA). Lead (II) acetate was purchased from Tianjin Huadong Chemical Reagent Company (Tianjin, China). All ULTRAPAGE-purified 15-mer TBA oligonucleotides (5′-GGTTGGTGTGGTTGG-3′) were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). Before use, the TBA sample was dissolved in 10 mM tris(hydroxymethyl)aminomethane-acetate acid (Tris-HAc) buffer solution of pH 7.2. The solution was

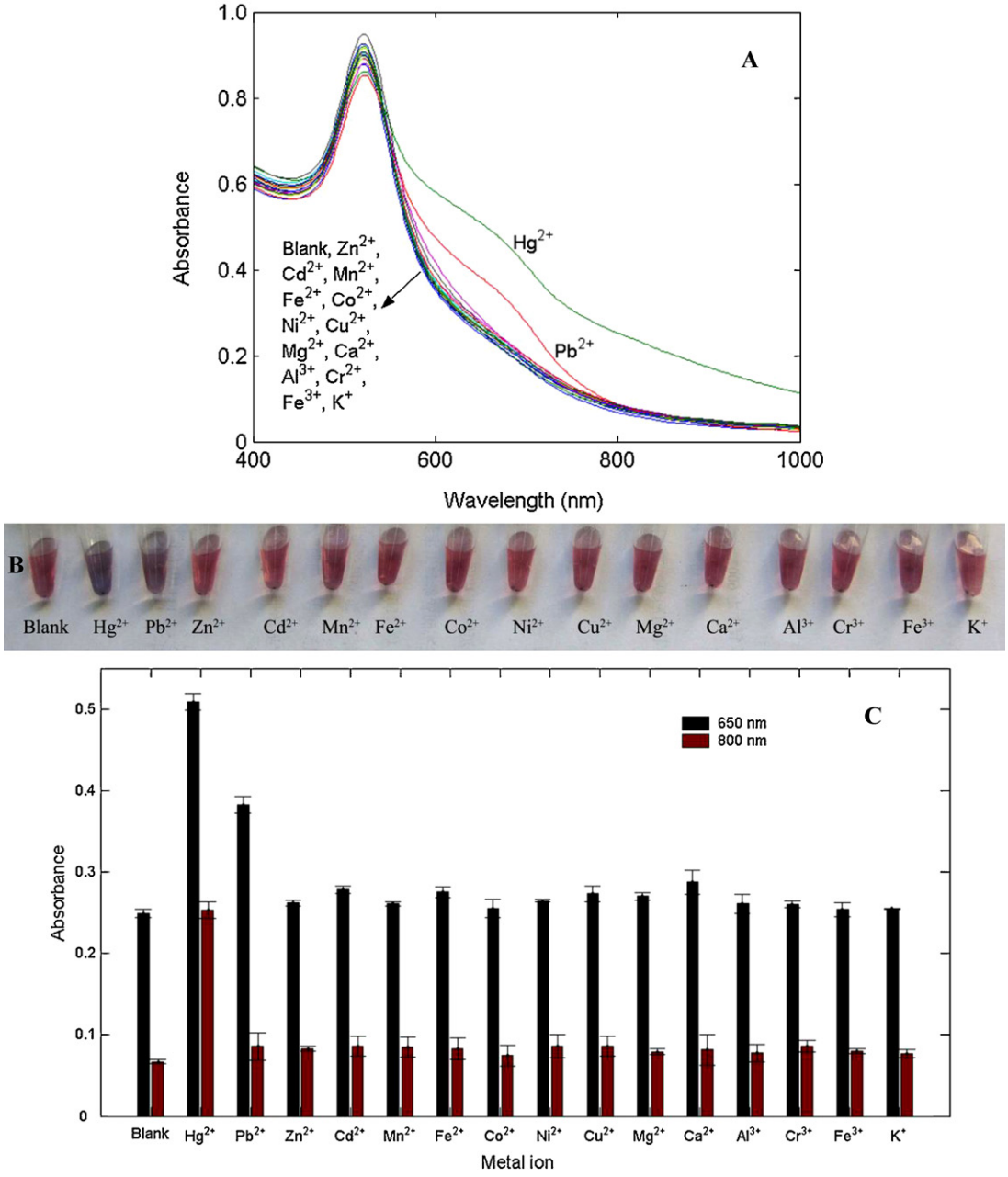

Fig. 1. (A) Absorption spectra of the AuNP solution mixed with 180 nM TBA in the absence (blank) or presence of 5.56 μM various metal ions after the addition of 40 mM NaCl. (B) Visual color changes upon treatment of the TBA/AuNP system with or without (blank) various metal ions in the presence of NaCl. Experimental conditions are as in (A). (C) Selectivity of the analysis of Hg^{2+} at 650 nm and 800 nm. Experimental conditions are as in (A).

heated to 95 °C for 5 min, cooled slowly to room temperature. The concentration of the oligonucleotide was determined by measuring the UV absorbance at 260 nm, and the molar extinction coefficients were calculated using a nearest neighbor approximation (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/). The synthesis of the citrate-protected AuNPs was described in Supplementary data. The concentration of the AuNPs was ~14 nM, which was determined according to the Beer's law by using the extinction coefficient of $2.01 \times 10^8\ M^{-1}\ cm^{-1}$ for 13 nm AuNPs in diameter at 520 nm (Maye et al., 2003). Unless specified, all other reagents were of analytical reagent grade and used without further purification or treatment. Ultrapure water (Milli-Q plus, Millipore Inc., Bedford, MA) was used throughout.

2.2. Instrumentation

Kinetic and spectral measurements were performed with the use of a Cary 50 UV–vis spectrometer (Varian, USA) with a 10 mm path length fused-silica cuvette at room temperature.

2.3. General procedure of colorimetric determination of Hg^{2+}

Into a 1.5 mL plastic vial, 13 μL of 9 μM TBA solution, an appropriate volume of water, 150 μL of the Tris–HAc buffer solution (10 mM, pH 7.2), and an appropriate volume of 50 μM Hg^{2+} or samples were micropipetted, mixed thoroughly and incubated for 10 min. Then, 135 μL of AuNP solution was added to give a volume of 426 μL, homogenized and allowed to stand for 5 min. Subsequently, 24 μL of 1 M NaCl was transferred to such solution to give a final volume of 450 μL and mixed thoroughly. After the solution was equilibrated for 15 min, 350 μL of the resulting solution was transferred to a 1.4 mL quartz cuvette. The UV–vis absorption spectrum was measured over the wavelength range from 400 nm to 1000 nm with respect to water. And a photograph was taken with Canon IXUS 110 IS digital camera. All assays were herein performed at room temperature.

2.4. Preparation of water samples

Water samples were collected from South Lake (Changchun, China). All the samples collected were spiked with a suitable amount of standard solution (500 μM) of Hg^{2+}, Pb^{2+} and other metal ions, mixed thoroughly, filtered through a 0.22 μm membrane, and then centrifuged for 15 min at 10,000 × *g*. A suitable volume of the as-prepared water samples was analyzed according to the proposed general procedure.

3. Results and discussion

3.1. Interaction of TBA/AuNPs/NaCl with various metal ions

Thymine-containing DNAs have specific molecular recognition functions towards Hg^{2+} (Miyake et al., 2006; Tanaka et al., 2007). Thus, this property has been recently explored to construct many label-free colorimetric Hg^{2+} biosensors based upon unmodified AuNPs (Li et al., 2008, 2009; Liu et al., 2008; Wang et al., 2008; Xu et al., 2009; Yu et al., 2009). However, some of these biosensors were often susceptible to severe Pb^{2+} interference (Li et al., 2008; Liu et al., 2008; Xu et al., 2009; Yu et al., 2009). Therefore, two important issues in the analysis of Hg^{2+} need to be discussed. One was the cause of Pb^{2+} interference, and the other is how to eliminate Pb^{2+} interference. For the former, it has been recently reported that thrombin-binding aptamer (TBA) containing six T units and nine guanosine (G) units with a random coil structure could be changed into a hairpin-like structure and a G-quadruplex structure upon the addition of Hg^{2+} and Pb^{2+} ions (Liu et al., 2009a; Smirnov and Shafer, 2000). Thus, we suppose that the Pb^{2+} interference in the analysis of Hg^{2+} mainly originated from the interaction between G bases in oligonucleotides and Pb^{2+}. To demonstrate the assumptions, TBA was selected as recognition elements in this work to reveal the conformation change of TBA upon the addition of Hg^{2+} and Pb^{2+}. Additionally, inspired by the observation of Li and Rothberg (Li and Rothberg, 2004), it was postulated that the unfolded TBA could be adsorbed onto citrate-coated AuNPs, and thus protect AuNPs against aggregation under high-salt conditions. However, in the presence of Hg^{2+} and/or Pb^{2+} ions, the random coil structure of the TBA could be changed into a relatively rigid conformation (hairpin-like structure and G-quadruplex structure). The rigid structure could prevent the exposure of the TBA bases to AuNPs, and thus could not be adsorbed onto AuNPs and lose the ability to protect AuNPs under high-salt conditions.

When the proof-of-concept experiments were performed, it could be clearly observed from Fig. 1A that there were a red shift and broadening in the surface plasmon resonance (SPR) absorption band of the AuNP solution containing TBA and Hg^{2+} or Pb^{2+} under high-salt conditions, while little spectral change occurred in the absence (blank) or presence of other various metal ions except Hg^{2+} and Pb^{2+}. Moreover, UV–vis absorption spectra of Hg^{2+}, Pb^{2+} and TBA showed no bands in the 400–1000 nm wavelength range, suggesting that they had no effect on the spectral change of AuNPs (data not shown). Correspondingly, Fig. 1B displays a visible color change from red to blue or purple after the addition of Hg^{2+} and Pb^{2+} to the TBA/AuNPs/NaCl solution, and no color change in the absence (blank) or presence of other metal ion. All results were well consistent with our assumptions, suggesting that Pb^{2+} interference originated from the conformation change of the TBA from random coil structure to G-quadruplex structure. Thus, the AuNP-based colorimetric biosensor showed high selectivity for both Hg^{2+} and Pb^{2+}. On the other hand, compared to that in the presence of Pb^{2+}, the absorption spectrum of TBA/AuNPs/NaCl in the presence of Hg^{2+} red shifted and broaden more pronounced, and has a characteristic absorption band over the wavelength range from 800 nm to 1000 nm. Therefore, the specificity of the system toward Hg^{2+} could be possibly improved by the selection of a characteristic wavelength of the aggregated AuNPs. Derived from Fig. 1A, the absorbance values at two different characteristic wavelengths (650 nm and 800 nm) of aggregated AuNPs were collected in the absence or presence of various metal ions (Fig. 1C). Clearly, the addition of Hg^{2+} or Pb^{2+} resulted in significant increases of absorbance

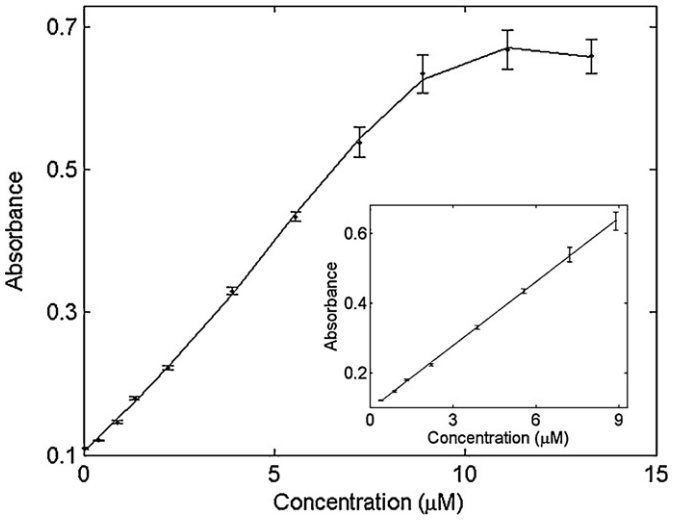

Fig. 2. Absorbance value at 800 nm versus Hg^{2+} concentration. Experimental conditions: c_{NaCl} = 53.3 mM and c_{TBA} = 260 nM. Inset: derived calibration curve.

Table 1
Determination of Hg^{2+} in water samples using the proposed method.

Samples	Mean found (μM)	Mean recovery[a] (%)	RSD[b] (%)
Hg^{2+} (1.0)[c], Pb^{2+} (0.2), Zn^{2+} (0.5), Mn^{2+} (0.5), Cr^{3+} (1.0)	1.04	104	1.0
Hg^{2+} (2.0), Pb^{2+} (3.0), Fe^{2+} (1.0), Co^{2+} (2.0), K^{+} (0.5), Al^{3+} (1.0)	2.09	105	0.5
Hg^{2+} (5.0), Pb^{2+} (5.0), Cd^{2+} (2.0), Fe^{3+} (1.5), Ni^{2+} (5.0)	5.00	100	0.2
Hg^{2+} (7.0), Pb^{2+} (1.0), Cu^{2+} (0.5), Mg^{2+} (1.5), Ca^{2+} (1.0), Al^{3+} (3.0)	6.90	99	0.2

[a] Mean recovery (%) = $100 \times (c_{\text{mean found}}/c_{\text{added}})$.
[b] Relative standard deviation of three determinations.
[c] Values in parentheses = concentration (μM) of the metal ion added.

at 650 nm, while other metal ions caused no such effect. In comparison, a considerable increase of absorbance at 800 nm was observed only in the presence of Hg^{2+}, indicating the efficient removal of interferences from all other metal ions including Pb^{2+} by the use of a characteristic wavelength of aggregated AuNPs. Subsequently, the level of Pb^{2+} interference was investigated using the proposed technique. The results showed that the absorbance change at 800 nm upon the addition of Pb^{2+} at concentrations up to 22.2 μM was less than 10% (data not shown). This demonstrated a tolerance limit to Pb^{2+} in the analysis of Hg^{2+} at a concentration of 22.2 μM.

3.2. Optimization of experimental conditions

In this work, the net absorbance value at 800 nm in the absence and presence of Hg^{2+} was used to optimize the experimental conditions. The net absorbance value was mainly influenced by the concentrations of NaCl and TBA. The effect of the concentration of NaCl in the range of 7–60 mM was first studied. The results showed that the net absorbance value increased substantially with the increase of the concentration of NaCl up to 53.3 mM. Hence, 53.3 mM NaCl was chosen for the experiments on the basis of higher sensitivity. Then, the effect of the TBA concentration in the range of 20–580 nM was investigated. It was found that the net absorbance value increased considerably as the concentration of TBA rose up to 260 nM. Thus, the concentration of 260 nM was selected to facilitate the experimental work.

3.3. Calibration model for Hg^{2+}

According to the above-mentioned general procedures, different concentrations of Hg^{2+} in the range of 0–13.3 μM were tested. As can be observed in Fig. 2, increasing concentrations of Hg^{2+} led to increase in absorbance at 800 nm until 11.1 μM. The concentration of Hg^{2+} over the range of 0.39–8.89 μM was employed to construct the calibration curve. The increased absorbance at 800 nm could be fitted as the equation of $A = 0.10 + 0.06c$ (Hg^{2+}, μM) with the correlation coefficient of 0.999. And the limit of detection (3σ) was estimated to be 200 nM, which compared well with those obtained from AuNP-based colorimetric assay with other T-containing oligonucleotides (Table S1, Supplementary data).

3.4. Application: determination of Hg^{2+} in water samples

The applications of the proposed method were evaluated for determination of Hg^{2+} in several water samples containing Hg^{2+}, Pb^{2+} and some other metal ions. The results are summarized in Table 1. As can be observed in Table 1, the mean recoveries of such samples were between 99% and 105%. The results reveal the potential application of this Hg^{2+} sensor in water samples.

4. Conclusion

This work investigates the interaction of thrombin-binding aptamer (TBA) containing six T units and nine guanosine (G) units with Hg^{2+} or Pb^{2+} using unmodified citrate-coated AuNPs as colorimetric signal readout. In the presence of Hg^{2+} or Pb^{2+}, TBA has a conformation alteration from a random coil structure to a hairpin or a quadruplex structure, respectively, and thus these conformation changes facilitate salt-induced AuNP aggregation. The results demonstrate that Pb^{2+} interference in the analysis of Hg^{2+} mainly originated from the interaction between G bases and Pb^{2+}. Compared to the previous studies using unmodified AuNPs and T-containing oligonucleotides (Table S1, Supplementary data), the work provides a promising alternative to Hg^{2+} determination in the presence of Pb^{2+} by the use of a characteristic wavelength of aggregated AuNPs instead of the universal masking agent of Pb^{2+} (2,6-pyridinedicarboxylic acid, PDCA) and this method is well applied for Hg^{2+} determination in water samples. In addition, this work further demonstrates that AuNPs as reporting probes are suitable to characterize the conformation change of oligonucleotides.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.01.014.

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