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Visual Detection of Hg²⁺ in Aqueous Solution Using Gold Nanoparticles and Thymine-Rich Hairpin DNA Probes

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Abstract

We report a sensitive method for visual detection of mercury ions (II) (Hg²⁺) in aqueous solution by using gold nanoparticles (Au-NPs) and thymine (T)-rich Hairpin DNA probes. The thiolated Hairpin DNA probe was immobilized on the Au-NP surface through a self-assembling method. Another thymine-rich, digoxin-labeled DNA probe was introduced to form DNA duplexes on the Au-NP surface with thymine-Hg²⁺-thymine (T-Hg²⁺-T) coordination in the presence of Hg²⁺. The Au-NPs associated with the formed duplexes were captured on the test zone of a lateral flow strip biocomponent (LFSB) by immunoreaction events between the digoxin on the duplexes and anti-digoxin antibodies on the LFSB. The accumulation of Au-NPs produced a characteristic red band on the test zone, enabling visual detection of Hg²⁺ without instrumentation. A detection limit of 0.1 nM was obtained under optimal experimental conditions. This method provides a simple, rapid, sensitive approach for the detection of Hg²⁺ and shows great promise for point-of-use and in-field detection of environmentally toxic mercury.

Keywords

Mercury; Hairpin; DNA; Visual detection; Gold nanoparticles

1. Introduction

Mercury toxicity in environmental pollution is a major concern because of increased usage of fossil fuels and agricultural products, both of which contain mercury which can cause long-term damage to biological systems (Dickerson et al., 2004). Mercury is known to disrupt biological events at the cellular level, to cause significant oxidative damage, and to be a carcinogen, so it poses a serious risk to endanger public health and the environment (Wang and Qian, 2006). The upper limit of Hg²⁺ mandated by United States Environmental Protection Agency (EPA) guidelines is 2 ppb (10 nM) in drinking water (U.S. EPA 2005). In

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addition to direct exposure to the metal's poisonous vapors, indirect exposure caused by eating mercury-tainted fish and other water-derived foods has also been fingered as a common route to the metal's toxic effects. The development of highly sensitive and selective methods for mercury detection is, therefore, of significant importance for the environment and for human health.

Conventional methods for mercury detection, such as atomic absorption spectroscopy (Jackson and Chen, 1996), selective cold vapor atomic fluorescence spectrometry (Leermakers et al., 2005), and inductively coupled plasma mass spectrometry (ICP-MS) (Berlingame et al., 1996), are widely used. Although they offer high accuracy, these methods require sophisticated and expensive instrumentation, skilled personnel, and time-consuming sample pretreatment, which are inappropriate for point-of-use and in-field applications. To overcome these drawbacks, a large number of sensors, including biosensors (Ono and Togashi, 2004), chemical sensors (Zhao et al., 2006), nanosensors (Chen et al., 2007), microcantilever sensors (Xu et al., 2002), and piezoelectric sensors (Manganiello et al., 2002), have been developed for the simple, rapid detection of Hg^{2+} in an aqueous solution. Although these methods have made great contributions toward the determination of Hg^{2+} , each of these sensors exhibits some features that limit its practical use, such as the poor aqueous solubility of some fluorophores, low-sensitivity, cross-sensitivity toward other metal ions, and the sophisticated synthesis of probe materials. Therefore, it is desirable to develop new methods or tools to overcome these limitations.

Because of the shorter measuring period, free of instrumentation and skilled personnel, colorimetric detection of Hg^{2+} has attracted considerable interest (Huang and Chang, 2007; Lee et al., 2007). In particular, newly developed colorimetric approaches, which combine the unique optical properties of gold nanoparticles (Au-NP) with the coordinate interaction between Hg^{2+} and bis-thymine of oligonucleotides, present an exciting and truly revolutionary approach for mercury detection (Lee et al., 2007; L. Li et al., 2009; T. Li et al., 2009). The approach is rooted in two well-established scientific foundations: the color change caused by the aggregation of Au-NPs and the fact that T-T mismatches of complementary sequences can bind the Hg^{2+} ions, which leads to the conformation change of a DNA probe or the formation of DNA duplexes. The later has also been widely used for the development of various Hg^{2+} sensors with electrochemical (Kong et al., 2009) and optical transducers (Guo et al., 2009; C. Liu et al., 2009; Wang et al., 2008; Wang and Liu, 2008; Gao et al., 2008). However, there are two major drawbacks for the T- Hg^{2+} -T based colorimetric Hg^{2+} detections: (1) low sensitivity with the current detection limits of most colorimetric approaches (more than 10 nM) and (2) difficulties distinguishing the color change at low concentrations of Hg^{2+} .

Recently, we reported a disposable lateral flow strip biocomponent (LFSB) based on oligonucleotide-functionalized Au-NPs for visual detection of nucleic acids (Mao et al., 2009), proteins (H. Xu et al., 2009), and cancer cells (G. Liu et al., 2009). Moreover, a biotin-labeled hairpin DNA, when conjugated to the Au-NPs, exhibited the ability of visually differentiating target DNA and one-base mismatched DNA (He et al., 2010). Herein, we present a highly selective and sensitive method for visual detection of Hg^{2+} that relies on hairpin DNA-conjugated Au-NPs and thymine- Hg^{2+} -thymine (T- Hg^{2+} -T) coordination chemistry.

Compared to several recently developed T- Hg^{2+} -T based colorimetric Hg^{2+} detection schemes, our proposed method possesses some remarkable features: (1) low background by using the thymine-rich hairpin DNA probe, which eliminates the nonspecific binding of DNA probes in the absence of Hg^{2+} ; and (2) high sensitivity to detect as low as 0.1 nM

Hg²⁺ without instrumentation. The promising properties of the method are reported in the following sections.

2. Materials and Methods

2.1 Apparatus

The Airjet AJQ 3000 dispenser, Biojet BJQ 3000 dispenser, Clamshell Laminator, and the Guillotine cutting module CM 4000 were from Biodot LTD (Irvine, CA). The DT1030 portable strip reader was purchased from Shanghai Goldbio Tech. Co., LTD (Shanghai, China).

2.2 Reagents

Streptavidin, H₂AuCl₄, sucrose, hydroxylamine, Tween 20, Triton X-100, trisodium citrate, deoxyadenosine triphosphate (dATP), bovine serum albumin (BSA) and sodium chloride-sodium citrate (SSC) Buffer 20×concentrate (pH 7.0), and phosphate buffer saline (0.01 M PBS, pH 7.4) were purchased from Sigma-Aldrich (St. Louis, MO). Tris(hydroxymethyl)aminomethane (Tris), metal salts, and all the other reagents were purchased from Sigma and were used without further purification. The Hg²⁺ solution with different concentrations was prepared by a diluting mercury atomic absorption solution (1000 ppm) in ultrapure (>18 MΩ) water. Glass fibers (GF000800), cellulose fiber sample pads (CFSP001700), laminated cards (HF000MC100), and nitrocellulose membranes (HFB18004 and HFB 24004) were purchased from Millipore (Billerica, MA). Thiol-modified hairpin oligonucleotides (HO), digoxin-labeled detection DNA probes, and biotin-labeled control DNA probes were obtained from Integrated DNA Technologies, Inc. (Coralville, IA) and had the following sequences: Hairpin probe: 5'-Thiol/MC6-D/ACACGCCATCAAGCTTTAACTCATAGTGGCGTGT-3' Detection probe: Probe 1 (3T): 5'-DigN/ACGCTCACTATGAGTTTTGCTTGA-3' Probe 2 (5T): 5'-DigN/ACGCTCACTTTGTGTTTTGCTTGA-3' Probe 3 (7T): 5'-DigN/TCGCTCTCTTTGTGTTTTGCTTGA-3' Probe 4 (8T): 5'-DigN/TCGCTCTCTTTGTGTTTTGCTTGT-3' Capture probe (for the control line): 5'-Biotin/ACGCTCACTATGAGTAGTTAAAGCTTGA-3'

All chemicals used in this study were analytical reagent grade. All other solutions were prepared with ultrapure (>18 MΩ) water from a Millipore Milli-Q water purification system (Billerica, MA).

2.3. Preparation of Hairpin Oligonucleotide-Au-NP (HO-Au-NP) Conjugates

A HO modified with a thiol at its 5' end was used to prepare HO-Au-NPs (Figure 1A). The thiolated Hairpin probe (1.5 OD) was added to 1 mL of the tenfold-concentrated Au-NP solution. After shaking at room temperature (RT) for 30 min, 60 μL of 14.1 μM dATP were added to the solution and shaken at RT for another 15 min; finally, 20 μL of 1% sodium dodecyl sulfate (SDS) were added to reach a final concentration of 0.01% to stabilize the HO-Au-NP conjugates, and 50 μL of 2 M NaCl were added slowly for a final concentration of 0.1 M to "age" the HO. The mixture was incubated for half an hour at RT and then put at 4°C for 6 h to continue increasing the stability of the conjugates. The excess reagents were removed by centrifugation for 13 min at 12,000 rpm; after discarding the supernatant, the red pellets were washed twice with 0.01 M PBS (pH 8.4); recentrifuged; and redispersed in 1 mL of an aqueous solution containing 20 mM Na₃PO₄ · 12H₂O, 0.25% Tween, and 10% sucrose; the mixture was stored at 4°C before further use.

2.4. Preparation of the Lateral Flow Strip Biocomponent (LFSB)

The LFSB consisted of four components: sample application pad, conjugate pad, nitrocellulose membrane, and absorbent pad (Mao et al., 2009). All components were mounted on a common backing layer (typically an inert plastic, e.g., polyester) using the Clamshell Laminator (Biodot, Irvin, CA). The sample application pad (17 mm × 30 cm) was made from glass fiber (CFSP001700, Millipore) and saturated with a buffer (pH 8.0) containing 0.25% Triton X-100, 0.05 M Tris-HCl, and 0.15 M NaCl. Then, the pad was dried at 37°C for 2 h and stored in desiccators at RT. The conjugate pad (8 mm × 30 cm) was prepared by dispensing a desired volume of HO-Au-NP conjugate solution onto the glass fiber pad with the Airjet AJQ 3000 dispenser and then drying it at RT. The test and control zones on the nitrocellulose membrane (25 mm × 30 cm) were prepared by dispensing the concentration of 8 mg mL⁻¹ anti-digoxin antibody (test zone) and streptavidin-biotinylated control DNA probe (control zone) solutions, respectively. To facilitate the immobilization of control DNA probes on the nitrocellulose membrane, streptavidin was used to react with the biotinylated control DNA probes to form the streptavidin-biotin DNA complexes. (Mao et al., 2009) The complexes were then dispensed on the control zone of nitrocellulose membrane with the Biojet BJQ 3000 dispenser. The distance between the test and control zones was 3 mm. The anti-digoxin and control DNA probe loaded membrane was then dried at 37°C for 1 h and stored at 4°C in a dry state. Finally, the sample pad, conjugate pad, nitrocellulose membrane, and absorption pad were assembled on a plastic adhesive backing (60 mm × 30 cm) using the clamshell laminator. Each part overlapped 2 mm to ensure that the solution was migrating through the strip during the assay. Strips with a 3-mm width were cut by using the Guillotin CM 4000 cutting module.

2.5. Analytical Procedure

Ten microliters of digoxin-labeled detection DNA were added to 90 µL of sample solution containing a desired concentration of Hg²⁺; the sample solution was prepared with a running buffer (10 mM Tris-HCl + 1.75 mM Mg²⁺ + 1/80 SSC, pH 8.0). The mixture was incubated for 10 min at RT and then applied to the sample pad of the LFSB. After waiting 10 min, an additional 60-µL running buffer was added to the LFSB sample pad. Accumulation of Au-NPs on the test and control zones produced the characteristic red bands. Visual detection of Hg²⁺ was simply realized by observing the color of the LFSB test zone. Two red bands (both on the test and control zones) indicated a “positive” result; one red band (on the control zone) indicated a “negative” result. The intensities of the red bands were recorded using the portable strip reader combined with the “AuBio strip reader” software which could search the red bands in a fixed reaction area automatically and then figure out parameters such as peak height and area integral.

2.6 Analysis of river water and tap water samples

River water sample was obtained from the Red River (Fargo, ND). Tap water sample was obtained from the Tap in our laboratory. All samples were first filtered through a 0.22-µm filter membrane to remove insoluble substances. For LFSB tests, 10 µL of digoxin-labeled DNA probe and 10 µL of buffer (0.1 M Tris-HCl + 17.5 mM Mg²⁺ + 1/8 SSC, pH 8.0) were added to 80 µL of water sample solution. Other procedures were the same as that described in 2.5 (*Analytical procedure*). The water samples were spiked with Hg²⁺ with different concentrations, and then tested with LFSB following the above procedure. The results were compared with that obtained by ICP-MS (PerkinElmer Inc.) using EPA method 6020A (7439-97-6).

3. Results and Discussion

3.1. Principle

The principle of Hg^{2+} detection is based on T- Hg^{2+} -T coordination chemistry and immuno-capturing Au-NPs on a LFSB; the protocol is illustrated in Figure 1. In this study, Au-NP is used as a tracer to monitor the Hg^{2+} coordination events between the thymine (T)-rich hairpin oligonucleotide (HO) and the digoxin-labeled detection DNA probe, which is complementary with part of the HO except eight T-T mismatches (detection probe 4, shown in red bold in **reagents section**). The HO was immobilized on the Au-NP surface by self-assembling via the Au-S bond; the leftover space of the Au-NP surface was blocked with dATP (Figure 1A). The HO-Au-NP conjugates were dispensed on the conjugated pad of the LFSB (Figure 1B). The sample solution containing Hg^{2+} was first mixed with digoxin-labeled detection DNA probes, and the mixture was incubated at RT for 10 min. Such incubation brought Hg^{2+} cations to the digoxin-labeled DNA because of the T- Hg^{2+} interaction. The mixture was then applied on the sample application pad (Figure 1B). The solution migrated by capillary action and passed the conjugate pad, and then rehydrated the HO-Au-NP conjugates. Because of the coordination of Hg^{2+} , the detection DNA probe bound with the HO on the Au-NP surface to form the duplex DNA on the Au-NP surface and continued to migrate along the strip. The Au-NPs associated with the formed duplexes were captured on the test zone of the LFSB by immunoreaction events between the digoxin associated with the duplexes and anti-digoxin antibodies, which were pre-dispensed on the LFSB test zone (Figure 1B). The accumulation of Au-NPs produced a characteristic red band on the test zone, enabling visual detection of Hg^{2+} without instrumentation (Figure 1C). The excess HO-Au-NP conjugates continued to migrate and passed the control zone where the control DNA probe (complementary with HO) was immobilized. Then, the excess HO-Au-NP conjugates were captured by the hybridization events between the HO and the control DNA probe, thus forming a second red band (Figure 1C). In the absence of Hg^{2+} , no red band was observed on the test zone. In this case, a red band on the control zone showed that the LFSB is working properly (Figure 1C). The LFSB provided a low-cost, easy-to-use and disposable tool to determine Hg^{2+} .

As a proof of concept experiment, a detection DNA probe containing 8 T-T mismatches with the designed HO was selected for the detection of Hg^{2+} . Figure 1D presents the typical photo images and corresponding optical responses of the LFSB in the absence (control) and presence of 10 nM Hg^{2+} . As anticipated, a bright red band was shown in the LFSB test zone in the presence of 10 nM Hg^{2+} ; no red band was observed on the LFSB test zone in the absence of Hg^{2+} . The red band that appeared on the LFSB test zone indicated that the formation of Hg^{2+} -mediated DNA duplexes brought digoxin on the Au-NP surface, which resulted in the accumulation of Au-NPs on the LFSB test zone through the immunoreactions events between the digoxin and pre-immobilized anti-digoxin antibody on the test zone. It should be noted that a high background signal (control) was observed when a straight-chain oligonucleotide probe, instead of a HO, was used to conjugate Au-NPs (results not shown). The low background signal from the HO probe would benefit from its unique ability for differentiating a single-base mismatched target and a complementary target.

3.2. Optimization of the Sequences and Concentration of the DNA Detection Probe

In the current study, the mechanism of detecting Hg^{2+} was based on T- Hg^{2+} -T coordination chemistry and immuno-capturing events. The response of Hg^{2+} on the LFSB relied on the amount of Au-NPs on the LFSB test zone which, in turn, corresponded to the amount of duplex DNA (T- Hg^{2+} -T) on the Au-NP surface in the presence of Hg^{2+} . Therefore, the number of T-T mismatches between the HO and DNA detection probe affected the response of Hg^{2+} on the LFSB. To this regard, we systematically studied the effect of the DNA

detection probe sequences on the LFSB response. Four DNA detection probes, which are complementary with the HO except for various numbers of T-T mismatches, were used to test the response of Hg^{2+} on the LFSB. The performance of the DNA detection probes was evaluated by comparing the signal-to-noise (S/N) ratio of the LFSB. Figure 2A presents the histogram of the LFSB S/N ratio using the DNA detection probes with different numbers of thymine. One can see that the S/N ratio increases with higher numbers of thymine. The increase in the S/N ratio at high numbers of T-T mismatches is ascribed to the decreased background signal which was caused by the reduced hybridization ability in the presence of more T-T mismatches for oligonucleotides in the absence of Hg^{2+} . Therefore, a DNA probe with eight thymines was used in the following studies. We also studied the effect of the DNA detection probe concentration on the S/N ratio of the LFSB (Figure 2B). It can be seen that the S/N ratio becomes stable after the DNA concentration is more than 10 nM, which was used for the following assays.

3.3. Incubation Time and Temperature Studies

In the present study, the digoxin-labeled DNA detection probe was pre-incubated with the sample solution prior to application on the sample pad. The incubation brought Hg^{2+} to the digoxin-labeled DNA detection probe because of the T- Hg^{2+} interaction and increased the coordination efficiency of T- Hg^{2+} -T on the LFSB, thus enhancing the detection method's sensitivity. Therefore, we studied the effect of incubation time and temperature on the Hg^{2+} response on the LFSB (see Figure S1 in the supporting information). We compared the S/N ratios of LFSBs for 50 nM Hg^{2+} with 6 different incubation times (5, 10, 15, 20, 25, and 30 min) at different temperatures (25°C, 37°C, and 45°C). It was found that the S/N ratio of LFSB at 25°C increased with more incubation time up to 10 min, and then, it saturated at longer incubation times. At higher incubation temperatures (37°C and 45°C), the S/N ratio decreased with a longer incubation time. The S/N ratio loss at a high temperature and long incubation time may be attributed to the confirmation change of HO under these conditions; the stem-loop of HOs on the Au-NP surface was opened and produced the straight-chain oligonucleotides which induced the nonspecific binding between the DNA detection probe and HO in the absence of Hg^{2+} , resulting in a high background signal. Therefore, an incubation time of 10 min at RT was used for the following experiments.

3.4. Running Buffer Effect

Another factor that affects the LFSB sensitivity and reproducibility is the use of various buffers. Appropriate buffers minimize the nonspecific adsorption, as well as increasing the efficiencies of T- Hg^{2+} -T coordination and immunoreactions, thus the sensitivity of the LFSB. The Tris-HCl- Mg^{2+} buffer has been used widely for T- Hg^{2+} -T coordination, and SSC is one of the best buffers for DNA hybridization reactions. In the current study, we tested the effect of buffer components on the S/N ratio of LFSB. Buffers including PB, SSC, Tris-HCl, Tris-HCl+ Mg^{2+} , and the mixture buffer of Tris-HCl + Mg^{2+} + SSC (Figure 3A) were used in the experiments. It was found that the highest S/N ratio was obtained with the buffer mixture containing Tris-HCl+ Mg^{2+} and SSC. The component concentrations of the Tris-HCl+ Mg^{2+} +SSC buffer were further optimized (see Figure S2 in the supporting information). The best result was obtained with the buffer containing 10 mM Tris-HCl, 1.75 mM Mg^{2+} , and 1/80 SSC.

3.5. Effect of the Conjugate Volume

In the current study, HO-Au-NPs were used as probes for monitoring T- Hg^{2+} -T coordination events. The Au-NP accumulations on the LFSB test and control zones were visualized as red bands which could be used for the visual detection of Hg^{2+} . The intensities of the red bands depended on the captured HO-Au-NP conjugates on the test and control zones which, in turn, corresponded to the amount of conjugates on the pad. To obtain a

maximum response using a minimal amount of HO-Au-NP conjugates, the HO-Au-NP on the conjugate pad was optimized by increasing the volume of HO-Au-NP conjugates loaded on the pad (Figure 3B). It was found that the S/N ratio of LFSB increased up to 5 μL ; a further volume increase caused a decrease of for S/N ratio, which was ascribed to increased nonspecific adsorption and an increased background signal; 5 μL of HO-Au-NP conjugates were routinely used for the following assays.

3.6. Effect of Anti-Digoxin Amount

Another factor considered for the assay optimization is the amount of anti-digoxin on the LFSB test zone. Anti-digoxin was used to capture Au-NPs by immunoreaction events between anti-digoxin and digoxin. The quantity of anti-digoxin affects the amount of captured Au-NPs and the LFSB sensitivity. Anti-digoxin with different concentrations, ranging from 2 mg mL^{-1} to 10 mg mL^{-1} , was dispensed on the LFSB test zone. The performances were evaluated by comparing the S/N ratios of the LFSBs (Figure 3C). One can see that the S/N ratio of LFSB increases upon raising the anti-digoxin concentration from 2.0 mg mL^{-1} to 8.0 mg mL^{-1} ; further increasing the anti-digoxin concentration leads to decreases of the S/N ratio, which may be caused by the increased nonspecific adsorption of HO-Au-NPs on the test zone. Therefore, an 8.0 mg mL^{-1} anti-digoxin was used to prepare the LFSB test zone.

3.7. Analytical Performances

Under optimal experimental conditions, we examined the performance of the LFSB with different concentrations of Hg^{2+} . Figure 4 presents the typical photo images of LFSBs in the presence of different of Hg^{2+} concentrations ranging from 0 nM to 200 nM. There was no distinct red band observed on the LFSB test zone in the absence of Hg^{2+} (control), indicating negligible nonspecific adsorption under the optimized experimental condition. The test-line band was quite visible, even at 0.1-nM Hg^{2+} which can be used as the threshold for the visual determination (yes/no) of Hg^{2+} without instrumentation. The threshold in this present work was below the maximum level of mercury permitted by the U.S. EPA for drinking water and was lower than most methods reported (Lee et al., 2007; Xue et al. 2008; L. Li et al., 2009; T. Li et al., 2009). In addition, quantitative detection was performed by recording the peak areas of the red bands in the test zone with the aid of a portable strip reader. The resulting calibration curve (Figure 4, right side) shows that the peak areas versus the logarithm of Hg^{2+} are linear over the 0.1 to 100 nM range and are suitable for quantitative work.

To assess the selectivity of the LFSB, other metal ions (Pb^{2+} , Zn^{2+} , Ni^{2+} , Cu^{2+} , Co^{2+} , Ag^{+} , Fe^{2+} , Pd^{2+} , and Cd^{2+}) at 1 μM , the mixtures of Hg^{2+} (10 nM) and the corresponding metal ions have been tested on the LFSBs (Figure 5A). Blue bars and red bars represent the individual metal ion's responses, and the mixture of Hg^{2+} and corresponding metal ions on the LFSB, respectively. Excellent selectivity for Hg^{2+} detection was achieved over alkali, alkaline earth, and heavy transition-metal ions. Certainly, the specific Hg^{2+} detection is mainly attributed to its ability to form stable T- Hg^{2+} -T complexes.

The sensitive and specific response was coupled with high reproducibility. The reproducibility of the LFSB was studied by testing the sample solutions at different concentration levels (0-nM, 0.5-nM, and 50-nM Hg^{2+} ; see the photo images of the LFSB in Figure S3 of the support information). Samples at the same concentration level were tested 6 times with 6 different LFSBs from the same batch preparation. One can see that similar responses were obtained at the same concentration level. The coefficients of variation (CV) of the signals for the control, 0.5-nM, and 50-nM target DNA were 5.7%, 6.3%, and 5.1%, respectively ($n = 6$). The overall relative standard deviation was less than 6.5%, which

indicates good reproducibility for the LFSB. The LFSB stability was investigated by storing the LFSBs at RT. Figure 5B presents the responses of LFSBs with 5 nM Hg^{2+} at different storage periods. It can be seen the response of LFSB decreased with the increase of storage time slowly. Their responses decreased by 80% of newly prepared LFSB after nine weeks' storage at RT indicating that the LFSB has good stability.

3.8. Assay of Hg^{2+} Concentrations in Water Samples

The applications of the proposed method were evaluated to determine Hg^{2+} in river water and tap water, and the results were validated with ICP-MS. Neither our method nor the ICP-MS system detected the presence of Hg^{2+} ions in both the river-water and tap water-samples. The water samples were thus spiked with Hg^{2+} at different concentrations. The results are summarized in Table 1 and show good agreement with the expected values. These results reveal the practicality of using our method for the determination of quantifying Hg^{2+} ions in environmental samples.

4. Conclusions

We developed a simple and sensitive method for visual detection of Hg^{2+} based on hairpin-probe modified Au-NP, T- Hg^{2+} -T coordination chemistry and a LFSB. Under optimal conditions, we can detect as low as 0.1-nM Hg^{2+} without instrumentation. The presence of other metal ions does not interfere with the detection of Hg^{2+} . The proposed method provides a simple, rapid, sensitive tool for the detection of Hg^{2+} , and this technique shows great promise for point-of-use and in-field detection of environmentally toxic mercury. Future work aims to develop the LFSB for multiplex visual detection of toxic metal ions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) Schematic illustration of the preparation of HO-Au-NP conjugates; (B) Schematic illustration of visual detection of Hg^{2+} on lateral flow strip biosensor; and (C) Visual detection of the sample solution with Hg^{2+} and without Hg^{2+} on the lateral flow strip biosensor; (D) Typical photo images and recorded optical responses of LFSBs in the absence (control) and presence of 10-nM Hg^{2+} . Assay time: 30 min.

Figure 2.

(A) Histogram of the S/N ratio for the LFSB using the DNA detection probes with different numbers of thymine and (B) Effect of the DNA detection probe concentration on the S/N ratio of the LFSB. Hg^{2+} concentration: 50 nM.

Figure 3.

(A) Effect of running buffer component on the S/N ratio of the LFSB; (B) Effect of HO-Au-NP conjugate amount on the S/N ratio of the LFSB. The conjugate amount was adjusted by changing the dispensing cycles of the conjugate on the pad; and (C) Effect of the amount of anti-digoxin on the S/N ratio of the LFSB. Hg^{2+} concentration: 50 nM; Assay time: 30 min.

Figure 4.

Photo images (left) of the LFSBs with different concentrations of Hg^{2+} and the resulting calibration curve (right). The photo images of the LFSB were recorded with a digital camera, and the optical responses of red bands on the LFSB were recorded with a strip reader. Assay time: 30 min. Running buffer: 10 mM Tris-HCl + 1.75 mM Mg^{2+} + 1/80 SSC.

Figure 5.

(A) The optical intensities (peak areas) of the LFSB test zone analyzed with 50 nM Hg^{2+} and 1 μM of other metal ions. Blue bar: individual metal ions in the sample solution; Red bar: the mixture of Hg^{2+} (50 nM) and a corresponding metal ion; (B) The optical intensities of the LFSB test zone with 5 nM Hg^{2+} at different storage times (weeks). Data for each storage period were obtained from six LFSBs and error bars indicate standard deviations.

Table 1Determination of Hg^{2+} ions in water samples using the proposed method and ICP-MS

Sample	Added [Hg^{2+}]/nM	Proposed method Mean ^a $\pm$ SD ^b /nM	ICPMS Mean $\pm$ SD/ nM
Tap water 1	2.0	1.98 $\pm$ 0.12	2.02 $\pm$ 0.05
Tap water 2	5.0	4.89 $\pm$ 0.31	4.95 $\pm$ 0.13
River Water 1	10.0	9.73 $\pm$ 0.29	9.88 $\pm$ 0.25
River Water 2	50.0	48.72 $\pm$ 0.50	49.13 $\pm$ 0.35

^a the mean of three determinations.^b SD=standard deviation