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Highly Selective DNA-Based Sensor for Lead(II) and Mercury(II) Ions

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We have developed a technique for the highly selective and sensitive detection of Pb^{2+} and Hg^{2+} using a thrombin-binding aptamer (TBA) probe labeled with the donor carboxyfluorescein (FAM) and the quencher 4-([4-(dimethylamino)phenyl]azo)benzoic acid (DABCYL) at its 5′ and 3′ termini, respectively. The TBA has a random coil structure that changes into a G-quartet structure and a hairpin-like structure upon binding Pb^{2+} and Hg^{2+} ions, respectively. As a result, the fluorescence decreases through fluorescence resonance energy transfer (FRET) between the fluorophore and quencher. These changes in fluorescence intensity allow the selective detection of Pb^{2+} and Hg^{2+} ions at concentrations as low as 300 pM and 5.0 nM using this TBA probe in the presence of phytic acid and a random DNA/NaCN mixture, respectively. The linear correlation existed between the fluorescence intensity and the concentration of Pb^{2+} and Hg^{2+} over the range of 0.5−30 nM (R^2 = 0.98) and 10−200 nM (R^2 = 0.98), respectively. To the best of our knowledge, this is the first example of a single DNA-based sensor that allows the detection of both Hg^{2+} and Pb^{2+} ions. This simple and cost-effective probe was also applied to separately determine Pb^{2+} in soil samples and spiked Hg^{2+} in pond samples.

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.[1] Lead and mercury are two of the most toxic metallic pollutants; for example, lead can cause renal malfunction and inhibit brain development[2] and mercury can damage the brain, heart, and kidneys.[3] Although the powerful technique of inductively coupled plasma mass spectrometry (ICPMS) is used in most current protocols for the detection of these two metal ions, it is rather expensive, complex, and not suitable for on-site analyses.[4] The past few years have witnessed great progress in the development of optical and electrochemical techniques for the detection of metal ions. Procedures using small molecules,[5] DNAzymes,[6] oligonucleotides,[7] polymers,[8] and functional nanoparticles[7c,9] have all been developed for the selective detection of Pb^{2+} and/or Hg^{2+}. For example, a fluorescence resonance energy transfer (FRET)-based DNAzyme system was demonstrated for Pb^{2+} sensing. The sensor was made of FRET between fluorophore and quencher labeled on the DNAzyme (17E) and its substrate, respectively. In the presence of Pb^{2+}, the 17E catalyzes hydrolytic cleavage of substrate and it turned on the fluorescence for sensing.[6] For the detection of Hg^{2+}, a colorimetric method was developed using DNA-modified gold nanoparticles (DNA−Au NPs) in aqueous media under a temperature control. In that assay, two types of DNA-functionalized Au NPs were prepared, each functionalized with different thiolated-DNA sequences (5′ HS−C_{10}−A_{10}−T−A_{10} 3′ and 5′ HS−C_{10}−T_{10}−T−T_{10} 3′), which are complementary except for a single thymidine−thymidine mismatch. Each increase in concentration of 1.0 μM results in an increase in the melting point by about 5.0 °C, thus providing an easy way of determining Hg^{2+} concentration.[9d] Nevertheless, many of these systems have limited practical use because of, for example, poor aqueous solubility, cross-sensitivity toward other metal ions, matrix interference, high cost (e.g., enzymes), complicated processing, the use of unstable molecules (e.g., RNA), or poor sensitivity. Previously, we unveiled a homoge-

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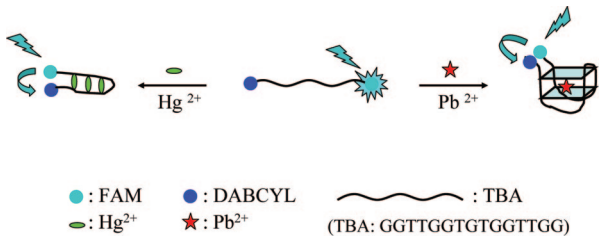
Scheme 1. Cartoon Representation of the Sensing Mechanism of the TBA Probe for the Detection of Hg^{2+} and Pb^{2+} Ions



neous assay using the DNA-binding dye TOTO-3 and polythymidine (poly-T) to detect Hg^{2+} through T−Hg^{2+}−T interactions, which induce a conformational change of poly-T into a folded structure that preferably binds TOTO-3.[7b] Although such optical (or electrochemical) sensing techniques can be sensitive and selective, they generally allow the detection of only one of the two metal ions.

In this paper we present a technique for the highly selective and sensitive detection of Pb^{2+} and Hg^{2+} using a thrombin-binding aptamer (TBA) probe[10,11] labeled with the donor carboxyfluorescein (FAM) and the quencher 4-([4-(dimethylamino)phenyl]azo)benzoic acid (DABCYL) at its 5′ and 3′ termini, respectively. The sensing mechanism of this probe is based on the change in the DNA strand's conformation from the linear to a folded structure upon binding the metal ions. These conformations exhibit different degrees of FRET between the fluorophore (donor) and quencher (acceptor) at the termini of each DNA probe.

EXPERIMENTAL SECTION

Chemicals. Acetic acid, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid hemisodium salt (HEPES), phytic acid, sodium cyanide (NaCN), tris(hydroxymethyl)aminomethane (Tris), and all of the metal salts used in this study were purchased from Aldrich (Milwaukee, WI). The TBA probe (Fam−5′-GGTTGGTGTGGTTGG-3′−DABCYL) and the random DNA (5′-ATGTACCGATCACTA-3′) were purchased from Integrated DNA Technology, Inc. (Coralville, IA). Montana Soil (SRM 2710) was obtained from the National Institute of Standards and Technology (NIST, Maryland). Milli-Q ultrapure water was used in each experiment. The toxic sodium cyanide was used with caution because it is a potent inhibitor of respiration.

Analysis of Samples. For lead sensing, aliquots (500 μL) of 10 mM Tris−acetate (pH 7.4) solutions containing the TBA probe (10 nM), Pb^{2+} (0−1.0 μM), NaCN (100 μM), and the random blocking DNA (100 nM) were equilibrated at room temperature for 15 min prior to measurement of the fluorescence. In this paper, the final concentrations of the species are provided. For mercury sensing, aliquots (500 μL) of 10 mM Tris−acetate (pH 7.4) solutions containing the TBA probe (10 nM), Hg^{2+} (0−1.0 μM), and phytic acid (100 μM) were equilibrated at room temperature for 15 min prior to measurement of the fluorescence using a fluorescence spectrophotometers (Cary Eclipse; Varian, California). The fluorescence lifetime of the TBA probe in the absence and presence of the two metal ions were measured using a Edinburgh FL 900 photon-counting system (Edinburgh, U.K.), and then the data were treated by fitting to a biexponential fluorescence decay. Acidic digestion of soil samples (1 g) was performed according to EPA method 305B.[12] Aliquots (50 μL) of the diluted soil samples (0.0001×) were spiked with standard solutions of Pb^{2+} over the concentration

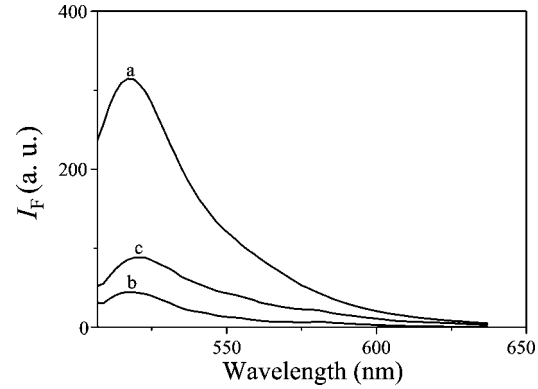


Figure 1. Fluorescence spectra of solutions of (a) TBA probe (10 nM) and (b and c) TBA probe (10 nM) in the presence of (b) Pb^{2+} (100 nM) and (c) Hg^{2+} (500 nM). Buffer, 10 mM Tris−acetate (pH 7.4); excitation wavelength, 475 nm. The fluorescence intensities (I_F) are plotted in arbitrary units (a. u.).

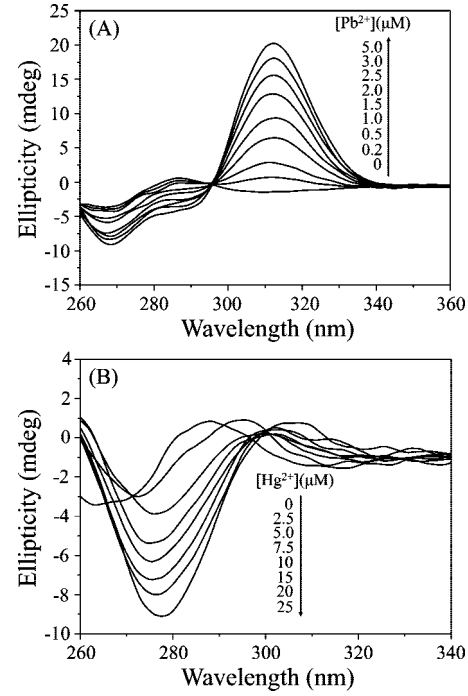


Figure 2. Ellipticity plotted with respect to the (A) Pb^{2+} ion concentration (0, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 5.0 μM) and (B) Hg^{2+} ion concentration (0, 2.5, 5.0, 7.5, 10, 15, 20, and 25 μM). Each sample was prepared in 10 mM Tris−acetate (pH 7.4) containing 5.0 μM TBA probe.

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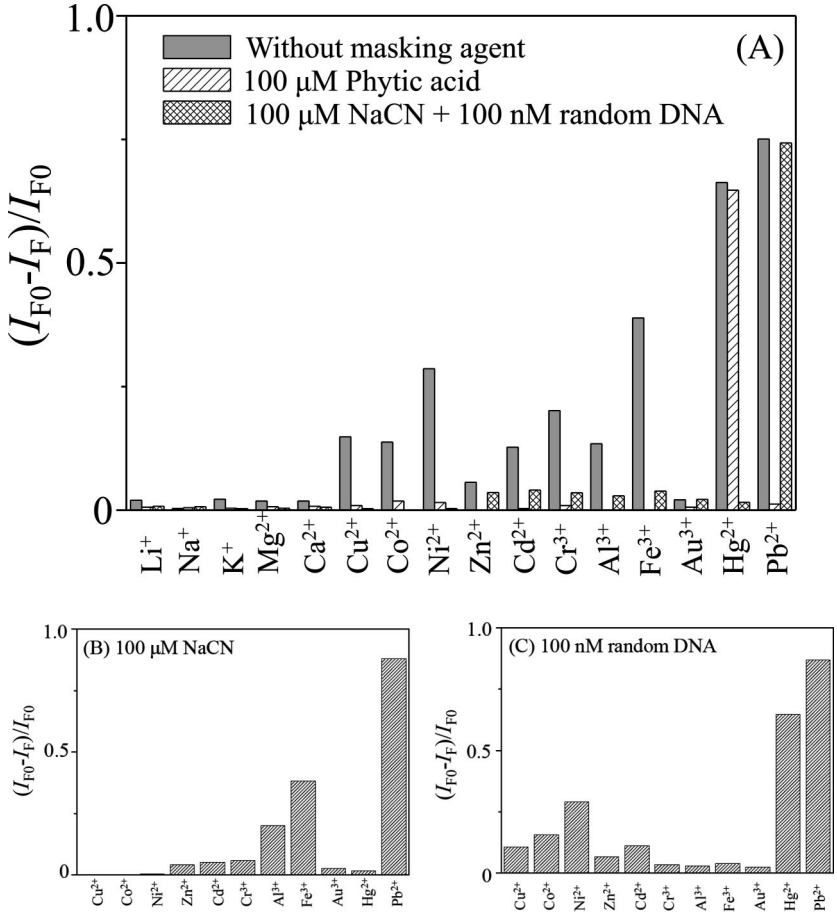

Figure 3. Quenching efficiency $(I_{F0} - I_F)/I_{F0}$ of the fluorescence intensity (518 nm) of the probe in the presence of metal ions in the absence and presence of masking agents. (A) Concentrations: Hg^{2+}, 1.0 μM; Pb^{2+}, 100 nM; Li^+ and Na^+, 100 μM; K^+, Mg^{2+}, and Ca^{2+}, 10 μM; other ions, 1.0 μM. (B and C) metal ions (1.0 μM) in the presence of NaCN (100 μM) and random DNA (100 nM), respectively.

range of 10−500 nM. Prior to analysis, the mixtures were diluted to 500 μL with 10 mM Tris−acetate (pH 7.4) solution containing the TBA probe (10 nM), NaCN (100 μM), and the random blocking DNA (100 nM). A water sample collected from a pond on the campus of National Taiwan University was filtered through a 0.2 μm membrane. Aliquots of the pond water (490 μL) were spiked with standard Hg^{2+} solutions (10 μL) at concentrations over the range of 10−500 nM. The spiked samples were then diluted to 1000 μL with 10 mM Tris−acetate (pH 7.4, 500 μL) containing 10 nM TBA probe and 100 μM phytic acid. The spiked samples were then analyzed separately using ICPMS and the present sensing technique.

RESULTS AND DISCUSSION

Sensing Strategy. Scheme 1 displays the sensing strategy of the TBA probe toward the two target metal ions (Pb^{2+} and Hg^{2+}). The TBA has a random coil structure that changes into a G-quartet structure[10] and a hairpin-like structure structure[11] upon binding Pb^{2+} and Hg^{2+} ions, respectively. The TBA consists of nine deoxyguanosine (G) units, which interact specifically with Pb^{2+} ions to form a G-quadruplex and six T units, which bind to Hg^{2+} ions through T−Hg^{2+}−T interactions. As a result of the decreased distance between the donor and acceptor moieties—and, hence, FRET between the FAM and DABCYL units—the fluorescence of FAM in the presence of Pb^{2+} or Hg^{2+} is weak. We performed proof-of-concept experiments for the detection of Pb^{2+} and Hg^{2+} ions using the TBA probe (10 nM). Curve a in Figure 1 indicates that the fluorescence of the solution containing the TBA probe was strong in the absence of the two metal ions, consistent with the fact that the TBA probe exists in a random coiled structure. After separately adding Pb^{2+} (100 nM) and Hg^{2+} (500 nM) to the probe solution, the fluorescence at 518 nm (excitation at 475 nm) decreased to different degrees, as curves b and c indicate, respectively. The greater decrease in fluorescence (i.e., greater FRET efficiency) in the presence of Pb^{2+} than that in the presence of Hg^{2+} reveals that the donor and acceptor are positioned relatively closer in the G-quartet structure. CD spectra (Figure 2) confirmed the formation of G-quadruplex and hairpin-like structure structures from the TBA probe in the presence of Pb^{2+} and Hg^{2+}, respectively.[10,11]

In order to further investigate the impacts of Pb^{2+} and Hg^{2+} on the fluorescence changes, we compared the lifetimes of the probe in the absence and presence of the metal ions. The lifetimes of the TBA, Hg^{2+}/TBA, and Pb^{2+}/TBA were 2.1, 1.1, and 0.8 ns, respectively. We also compared the fluorescence quenching efficiencies of the probe separately in the presence of Pb^{2+} and Hg^{2+} at the same concentration. The quenching efficiencies of Pb^{2+} and Hg^{2+} (both at 500 nM) were 81% and 66%, respectively. The results reveal that the distance of the two fluorophores in the TBA in the presence of Pb^{2+} was closer than that of Hg^{2+}.

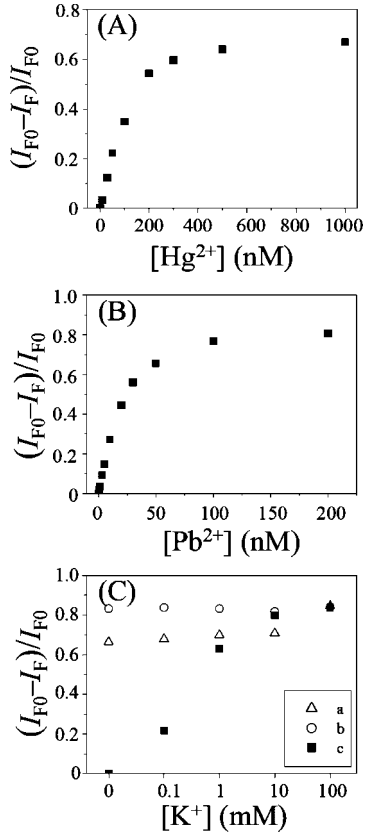

Figure 4. Quenching efficiency plotted with respect to the (A) Hg^{2+}, (B) Pb^{2+}, and (C) K^+ ion concentrations. The solution contained K^+ and interference metal ions (Pb^{2+}, 100.0 nM; Hg^{2+}, 500.0 nM) in the presence of (a) phytic acid (100 μM), (b) NaCN (100 μM) and random DNA (100 nM), and (c) phytic acid (100 μM), NaCN (100 μM), and random DNA (100 nM).

Buffer Composition, Masking Agents, and Selectivity. We carefully tested the detection of Hg^{2+} and Pb^{2+} using our probe in four different buffer systems (10 mM, pH 7.4), including Tris−acetate, HEPES, sodium acetate, and sodium phosphate. The values of $[(I_{F0} - I_F)/I_{F0}]$ for the solutions buffered with sodium phosphate, HEPES, Tris−acetate, and sodium acetate at 1.0 μM Pb^{2+} were 0.25, 0.78, 0.80, and 0.81, respectively. Herein, I_F and I_{F0} represent fluorescence intensity in the presence and absence of the metal ion, respectively. The values of $[(I_{F0} - I_F)/I_{F0}]$ for the solutions buffered with sodium phosphate, HEPES, Tris−acetate, and sodium acetate at 1.0 μM Hg^{2+} were 0.68, 0.66, 0.69, and 0.68, respectively (data not shown). The sensitivity decreased upon increasing the stability of metal−anion complexes; for example, we obtained lower sensitivity for Pb^{2+} in phosphate buffer than that in Tris−acetate buffer (formation constants: $pK_f = 43.5$ for Pb_3L_2 (L, phosphate) and $pK_f = 4.1$ for PbL_2 (L, acetate; $pK_f = 10.1$ for HgL_2).

To investigate the selectivity of the TBA probe toward these two metal ions, we added 100 nM Pb^{2+} and Hg^{2+}, 100 μM Li^+ and Na^+, 10 μM K^+, Mg^{2+}, and Ca^{2+}, and 100 nM Cu^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Cr^{3+}, Al^{3+}, Fe^{3+}, and Au^{3+} separately into the probe solutions. Only Pb^{2+} and Hg^{2+} caused decreases in the fluorescence of FAM (data not shown), revealing that the probe is selective for Pb^{2+} and Hg^{2+} ions. High concentrations (>1.0 μM) of Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Cr^{3+}, Al^{3+}, and Fe^{3+} ions, however, decreased the fluorescence of FAM, leading to false-positive signals (Figure 3A). To overcome this problem, we tested the effects of several masking reagents, including phytic acid, CN^-, and a random DNA strand. It is well-known that phosphate ions form stable complexes with several metal ions, including Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Pb^{2+}. Thus, we tested the masking capability of phytic acid for the interfering metal ions in our sensing system. Gratifyingly, only Hg^{2+} caused a decrease in the fluorescence of FAM in the presence of 100 μM phytic acid (Figure 3A); i.e., this sensing system is specific to Hg^{2+}. For example, in 10 mM Tris−acetate solution (pH 7.4) containing 100 μM phytic acid, the TBA probe provided high selectivity (50-fold or more) toward Hg^{2+} ions over the other metal ions. To improve the selectivity of the TBA probe toward Pb^{2+}, we added CN^- as a masking agent because it forms a much more stable complex with Hg^{2+} ions ($\log \beta_2 = 32.8$) than with Pb^{2+} ions.[13] As indicated in Figure 3B, the presence of CN^- masked the TBA probe toward other ions but not toward Pb^{2+}, Al^{3+}, or Fe^{3+}. To mask the two trivalent metal ions, we added a random DNA sample (100 nM) having the sequence 5′-ATGTACCGATCACTA-3′. Figure 3C reveals that the random DNA did not mask Pb^{2+}, but it did mask the Al^{3+} and Fe^{3+} ions by at least 78% and 90%, respectively. The random DNA interacted with the two interfering metal ions through electrostatic attractions. The interactions between Pb^{2+} and the random DNA are much weaker than those with TBA (G-quadruplex). In 10 mM Tris−acetate solution (pH 7.4) containing 100 μM NaCN and 100 nM of the random DNA, the TBA probe provided high selectivity (400-fold or more) toward Pb^{2+} ions over all of the other tested interference ions (Figure 3A).

Different possible interferences including anions and thiol compounds were also tested. We found that Cl^-, Br^-, F^-, NO_3^-, SO_4^{2-}, and CO_3^{2-} (all sodium salts), respectively, did not interfere the detection of Pb^{2+} and Hg^{2+}. However, when the concentration of NaCl increased from 10 to 100 mM, the value of $[(I_{F0} - I_F)/I_{F0}]$ for the solution increased from 0.02 to 0.25, mainly due to the formation of G-quartet under the high-salt conditions. We also found that thiol compounds, such as sodium sulfide, potassium peroxydisulfate, ethanethiol, and thiolurea at high concentrations caused interferences in sensing Pb^{2+} and Hg^{2+}. The tolerance concentration of these four thiol compounds for detecting Pb^{2+} and Hg^{2+} were all 100 and 10 μM, respectively. The tolerance concentration is defined by 50% decreases in the intensity of $(I_{F0} - I_F)/I_{F0}$ when the interference is present.

Sensitivity and Application. Under the optimal conditions, we investigated the sensitivity of the TBA probe toward Hg^{2+} and Pb^{2+} individually. The fluorescence of FAM decreased upon increasing the concentration of Hg^{2+} (Figure 4A) or Pb^{2+} (Figure 4B). We obtained linear responses of the expression $(I_{F0} - I_F)/I_{F0}$ against the concentrations of Hg^{2+} and Pb^{2+} over the ranges of 10−200 nM ($R^2 = 0.98$) and 0.5−30 nM ($R^2 = 0.98$), respectively. The TBA probe provided limits of detection (LODs) for Hg^{2+} and Pb^{2+} ions (signal-to-noise ratio = 3) of 5.0 nM and 300 pM, respectively. Thus, this approach provides a sensitivity toward Pb^{2+} ions that is more than 1 order of magnitude lower than that reported when using a DNAzyme.[6]

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In addition, this TBA probe provides a sensitivity for Hg^{2+} ions that is comparable to that of a system we reported previously.[7b,9c] Our results suggested that this TBA probe would be sensitive for monitoring the levels of Hg^{2+} and Pb^{2+} ions in foodstuffs and environmental samples. For example, the U.S. Food and Drug Administration suggests an action level for lead of 2.5 μM (500 ppb) in products intended for children; the U.S. Environmental Protection Agency permits the maximum level of mercury in drinking water to be 10 nM (2.0 ppb). In addition, we also demonstrated that our sensor provides selective detection of K^+ in the presence masking agents, phytic acid, NaCN, and random DNA (Figure 4C). The linear range of fluorescence response of our sensor for K^+ was about 0.1−10 mM.

Thus, we used our TBA probe to determine the concentrations of Pb^{2+} and Hg^{2+} in soil and pond water samples, respectively. The concentrations of lead determined ($n = 5$) using our new approach and ICPMS were 5.28 (±0.07) and 5.05 (±0.10) mg/g, respectively. The *F*-test value for the correlation between the two methods was 2.04 (the *F*-test value is 6.39 at a 95% confidence level), suggesting that the two methods did not differ significantly. We obtained a linear correlation ($R^2 = 0.97$) between the responses and the concentration of Hg^{2+} ions spiked into the pond water over the range of 10−200 nM. The TBA probe provided recoveries of 95−104% for these measurements. Neither our probe nor the ICPMS system detected the presence of Hg^{2+} ions in the pond water sample. These results reveal the practicality of using our TBA probe for the determination of Hg^{2+} and Pb^{2+} ions in environmental samples.

CONCLUSION

In conclusion, we have devised a new assay for the sensitive and selective detection of Hg^{2+} and Pb^{2+} ions using an aptamer (TBA) that interacts specifically with thrombin. Changes in the DNA strand's conformation allowed us to readily detect Hg^{2+} and Pb^{2+} using this TBA probe in the presence of phytic acid and a random DNA/NaCN mixture, respectively. The practicality of this method has been validated by the analyses of soil and water samples. To the best of our knowledge, this is the first example of a single DNA-based sensor that allows the detection of both Hg^{2+} and Pb^{2+} ions. The simple, rapid, and cost-effective sensing systems hold great practical for detection of heavy metal ions in real samples.

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