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Oligonucleotide-Based Fluorescence Probe for Sensitive and Selective Detection of Mercury(II) in Aqueous Solution

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In this paper we unveil a new homogeneous assay—using TOTO-3 and the polythymine oligonucleotide T_{33}—for the highly selective and sensitive detection of Hg^{2+} in aqueous solution. The fluorescence of TOTO-3 is weak in the absence or presence of randomly coiled T_{33}. After T_{33} interacts specifically with Hg^{2+} ions through $T-Hg^{2+}-T$ bonding, however, its conformation changes to form a folded structure that preferably binds to TOTO-3. As a result, the fluorescence of a mixture of T_{33} and TOTO-3 increases in the presence of Hg^{2+}. Our data from fluorescence polarization spectroscopy, capillary electrophoresis with laser-induced fluorescence detection, circular dichroism spectroscopy, and melting temperature measurements confirm the formation of folded $T_{33}-Hg^{2+}$ complexes. Under optimum conditions, the TOTO-3/T_{33} probe exhibited a high selectivity ($\geq$265-fold) toward Hg^{2+} over other metal ions, with a limit of detection of 0.6 ppb. We demonstrate the practicality of this TOTO-3/T_{33} probe for the rapid determination of Hg^{2+} levels in pond water and in batteries. This approach offers several advantages, including rapidity (<15 min), simplicity (label-free), and low cost.

Heavy-metal pollution is an important environmental issue because of its adverse effect on human health.[1] Mercury(II) is one of the most potently toxic metal ions; it affects many different areas of the brain and their associated functions, resulting in symptoms such as tremors, vision problems, deafness, and losses of muscle coordination, sensation, and memory.[2] In addition to the brain, inorganic mercury can damage the heart, kidney, stomach, and intestines.[3] The U.S. Environmental Protection Agency (EPA)'s estimate of annual total global mercury emission from all sources—both natural and human-generated—is ca. 7500 tons per year.[4] Thus, techniques for the detection and/or removal of Hg^{2+} are required to protect our environment and health.

Although many techniques such as atomic absorption/emission spectroscopy, inductively coupled plasma mass spectrometry (ICP-MS), and selective cold vapor atomic fluorescence spectrometry have been applied widely to detect Hg^{2+} in environmental samples, they require expensive and sophisticated instrumentation and/or complicated sample preparation processes[5] and their complexity makes them unsuitable for use in in-field Hg^{2+} analyses. Alternative techniques based on fluorescent probes using small molecules,[6] DNAzymes,[7a] oligonucleotides,[7b] polymer—protein complexes,[7c] and nanoparticles[7d–g] have been demonstrated for the detection of Hg^{2+}. Nevertheless, each of these approaches exhibits some feature that limits its practical use, be it poor aqueous solubility, cross-sensitivity toward other metal ions, short emission wavelengths, and/or weak fluorescence intensities.

In this study, we developed a simple and rapid fluorescence approach—using the polythymine oligonucleotide T_{33} and a double-strand-chelating dye TOTO-3—for the sensitive and selective detection of Hg^{2+} in aqueous solutions. TOTO-3 is a weakly fluorescent unsymmetrical cyanine dye that exhibits a more than 1000-fold enhancement in its fluorescence upon binding to double-stranded DNA, with excitation and emission wavelengths centered at 620 and 660 nm, respectively.[8] Our approach toward sensing

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Hg^{2+} is based on the fluorescence increase that occurs as a result of the strong interaction between TOTO-3 and the folded T_{33} structure induced by Hg^{2+}.

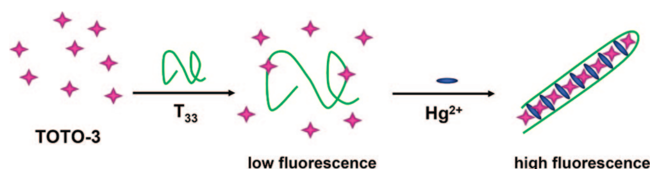
EXPERIMENTAL SECTION

Chemicals. Tris(hydroxymethyl)aminomethane (Tris), the metal salts, and all the other reagents were purchased from Aldrich (Milwaukee, WI). OliGreen, TOTO-3, and YOYO-3 were obtained from Molecular Probes (Portland, OR). The *N*,*N*-dimethyl-2,7-diazapyrenium dication was synthesized and purified according to an established procedure.[9] All of the DNA samples (T_7, T_{15}, T_{33}, T_{50}, T_{80}, and 6-FAM-T_{33}) were purchased from Integrated DNA Technology (Coralville, IA).

TOTO-3/T_{33}-Based Sensor for Hg^{2+}. A stock solution of TOTO-3 (0.2 μM) was prepared in DI water. Aliquots of this TOTO-3 solution (50 μL) were added separately to 5 mM Tris−HCl (pH 7.4) solutions containing Hg^{2+} (0–10 μM) and the unmodified T_{33} oligonucleotide (10 nM) to give final volumes of 500 μL. After equilibration at ambient temperature for 15 min, the fluorescence intensities of the mixtures were measured using a Cary Eclipse fluorescence spectrophotometer (Varian, CA), with excitation at 620 nm.

Capillary Electrophoresis (CE) and Optical Measurements. A homemade CE separation system was employed to monitor the Hg^{2+}-induced conformational changes of T_{33}. Briefly, a high-voltage (HV) power supply (Gamma High Voltage Research, Ormond Beach, FL) was used to drive the electrophoresis process. The entire detection system was enclosed in a black box equipped with an HV interlock. The HV end of the separation system was housed in a Plexiglass box for safety. A 10.0 mW laser having an output at 475 nm (B&W TEK, Inc., Newark, DE) was used for excitation. The emission light was collected with a 10× objective (numeric aperture 0.25). A 520 nm long-pass filter was used to block scattered light before the emitted light reached the phototube (R928, Hamamatsu Photonics K. K., Shizuoka-Ken, Japan). The fluorescence signal was transferred directly through a 10 kΩ resistor to a 24 bit A/D interface at 10 Hz (Borwin, JMBS Developments, Le Fontanil, France) and stored in a PC. Capillaries (i.d., 75 μm; o.d., 365 μm; total length, 40 cm; effective length, 30 cm; Polymicro Technologies, Phoenix, AZ) were dynamically coated overnight with 5.0% poly(vinyl pyrrolidone) (PVP; M_w 1.3 × 10^6) and then with 0.5% poly(ethylene oxide) (PEO; M_w 8.0 × 10^6) for 12 h prior to use in DNA separations. Before conducting CE separations, aliquots of Hg^{2+} (0–1.0 μM) were added separately to 5 mM Tris−HCl solutions (pH 7.4) containing 6-FAM-T_{33} (10 nM) and fluorescein (30 nM) and equilibrated for 10 min. The mixtures were injected hydrodynamically at the cathode end into the capillary at a 20 cm height for 10 s; separations were conducted at −5 kV. After each run, the capillary was sequentially washed and filled with 5 mM Tris−HCl (pH 7.4) containing Hg^{2+} (0–1.0 μM). For measurement of the fluorescence polarization spectra, 5 mM Tris−HCl solutions (pH 7.4) containing 6-FAM-T_{33} (50 nM) and Hg^{2+} (0–120 μM) were reacted for 15 min and then the anisotropy of each solution was recorded using a Cary Eclipse fluorescence spectrophotometer (Varian, CA) equipped with a manual polarizer accessory (Varian, CA). Circular dichroism (CD) spectroscopy measurements were conducted using a J-815 spectropolarimeter (JASCO, Inc., Easton, MD). Melting temperature (T_m) was determined by measuring the fluorescence intensities of the mixtures of TOTO-3 and T_{33} in the absence and presence of Hg^{2+}. We define the temperature at which the fluorescence of TOTO-3 reaches 50% of its original value as T_m.

Scheme 1. Schematic Representation of the Function of a Hg^{2+} Sensor That Operates based on Modulation of the Fluorescence of the Complex Formed between TOTO-3 and T_{33}



Analysis of Real Samples. A pond water sample from the National Taiwan University campus was filtered through a 0.2 μm membrane and analyzed using ICP-MS. Aliquots of the pond water (250 μL) were spiked with standard solutions (50 μL) containing Hg^{2+} at concentrations over the range of 0.01–1.0 μM. Next, 50 mM Tris−HCl solution (pH 7.4, 50 μL), T_{33} solution (100 nM, 50 μL), TOTO-3 solution (200 nM, 50 μL), and water (50 μL) were added to the mixtures to give final volumes of 500 μL. The mixtures were equilibrated for 15 min. The samples from three button-type alkaline manganese batteries were prepared according to the standard method published by the National Electrical Manufacturers Associations.[10] Briefly, these samples were digested in a mixture of HCl and HNO_3 (2:1, v/v) for 18 h and then directly basified through the addition of 0.5 N NaOH and filtered through a 0.2 μm membrane. The solutions (10 μL) were then diluted to 10 mL with 5 mM Tris−HCl solution (pH 7.4) prior to analysis using both ICP-MS and the TOTO-3/T_{33} probe. The quantitation of Hg^{2+} in these samples was obtained by applying a standard addition method.

RESULTS AND DISCUSSION

Sensing Strategy. Scheme 1 depicts the mechanism underlying this TOTO-3/T_{33} probe's approach to sensing Hg^{2+}. In the absence of Hg^{2+}, T_{33} exists in aqueous solution in a random-coil structure. Because the interactions between the randomly coiled T_{33} and TOTO-3 are weak, the fluorescence of such a mixture is weak. In the presence of Hg^{2+}, however, T−Hg^{2+}−T bonding results in T_{33} changing its random-coil conformation to that of a folded structure. Because TOTO-3 has a high affinity (K_b = ca. 10^9 M^{-1}) for double-stranded DNA (dsDNA), TOTO-3·DNA complexes form preferably in the presence of Hg^{2+}.[11] As a result of reduced collision and forming stiffer structures, TOTO-3·DNA complexes fluoresce more strongly than does free TOTO-3. Thus,

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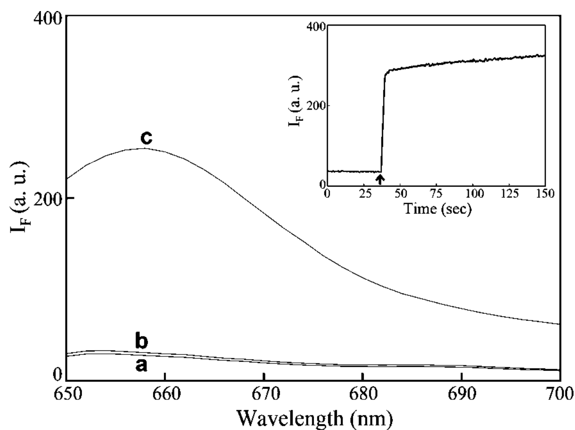

Figure 1. Fluorescence spectra of (a) TOTO-3 solution (20 nM) and (b and c) mixtures of TOTO-3 (20 nM) and T_{33} (10 nM) in the (b) absence and (c) presence of Hg^{2+} (1.0 μM). Inset: Temporal change in the fluorescence intensity (660 nm) of the mixture of T_{33} and TOTO-3 after the addition of Hg^{2+}. The arrow indicates the initial time at which Hg^{2+} was added. Background solution, 5 mM Tris−HCl (pH 7.4); excitation wavelength, 620 nm. The fluorescence intensities (I_F) are plotted in arbitrary units (a. u.).

the fluorescence intensity of the complexes formed between TOTO-3 and T_{33} increases in the presence of Hg^{2+}.

Evidence for Folded T_{33} Forming in the Presence of Hg^{2+}. Curve a in Figure 1 indicates that the fluorescence at 660 nm of TOTO-3 (20 nM) in 5 mM Tris−HCl solution (pH 7.4) is very weak when excited at 620 nm. The fluorescence intensity of TOTO-3 in the presence of 10 nM T_{33} is slightly higher (curve b), supporting the notion that it interacts weakly with random-coil T_{33}. After adding 1.0 μM Hg^{2+} to this mixture of TOTO-3 and T_{33}, a rapid (<10 s) and significant increase (8.3 fold) in fluorescence intensity occurred (curve c). In the absence of T_{33}, we did not observe any change in the fluorescence spectrum of TOTO-3 (20 nM) after adding Hg^{2+} (1.0 μM). To confirm the specificity of T_{33} toward Hg^{2+}, we tested a control DNA sample having the sequence GCC TTA ACT GCA GTA CTG GTG AAA TTG CT. We expected this control DNA to have difficultly folding in the presence of Hg^{2+}, mainly because it has a less of a chance to form T−Hg^{2+}−T bonds than does T_{33}. We observed only slight changes (<10%) in the fluorescence intensity of the mixture of TOTO-3 and the control DNA after adding the same amount of Hg^{2+}.

We further applied CE to support the notion of the formation of folded T_{33} in the presence of Hg^{2+}. Because the fluorescence of mixtures of TOTO-3 and random-coil T_{33} is weak, we could not observe random-coil T_{33} when using CE in conjunction with laser-induced fluorescence (LIF) detection. In other words, the changes in the electrophoretic mobility of the complexes of T_{33} and TOTO-3 in the presence of Hg^{2+} were difficult to observe using CE-LIF. Thus, we use a 5′ end labeled 6-FAM-T_{33} for the CE experiment. Upon increasing the concentration of Hg^{2+}, the migration time for 6-FAM-T_{33} decreased, while that for the internal standard (fluorescein) remained almost unchanged (Figure 2A). We suspect that once 6-FAM-T_{33} interacted with Hg^{2+}, its conformation changed from a random coil to a folded structure leading to increased electrophoretic mobility (i.e., a decrease in migration time).[12] On the basis of the plot in Figure 2B, we calculated the binding constant (K_b) for the interaction between T_{33} and Hg^{2+} to be 6.1×10^6 M^{-1}. For comparison, we note that a value of K_b of 4.2×10^5 M^{-1} has been reported for Hg^{2+} interacting with two oligonucleotides having two binding sites for Hg^{2+} (i.e., each has two T units).[13]

The formation of folded DNA molecules having many T residues in the presence of Hg^{2+} has been proven using CD and

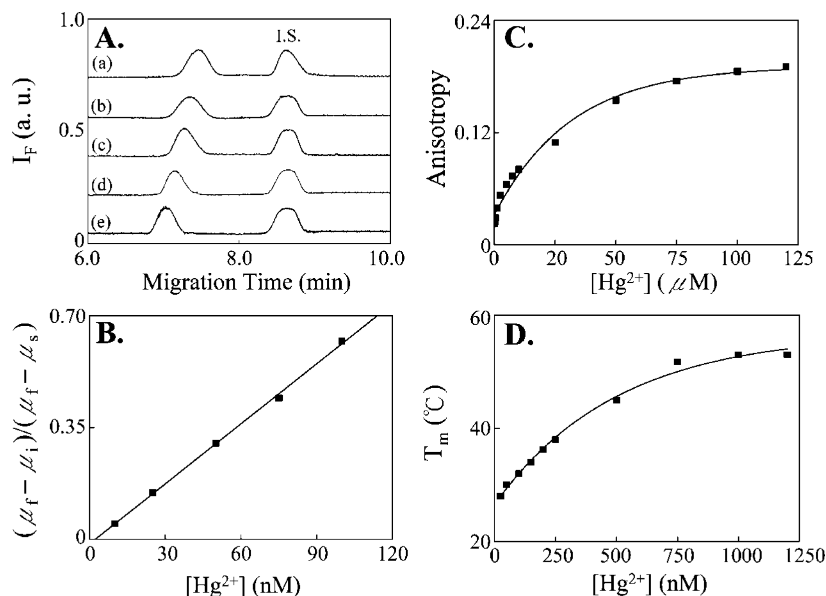

Figure 2. (A) Electropherograms of a solution containing 6-FAM-T_{33} (10 nM), fluorescein (30 nM), and Hg^{2+} at concentrations of (a) 0, (b) 50, (c) 100, (d) 250, and (e) 500 nM. (B) Plot of the relative mobility difference $(\mu_f - \mu_i)/(\mu_f - \mu_s)$ versus the concentration of Hg^{2+}, where μ_f, μ_s, and μ_i are the electrophoretic mobilities of 6-FAM-T_{33} (10 nM) in the absence of Hg^{2+}, in the presence of 1.0 μM Hg^{2+}, and in the presence of 10–100 nM Hg^{2+}, respectively. Background electrolytes: 5 mM Tris−HCl (pH 7.4) containing (a) 0, (b) 50, (c) 100, (d) 250, and (e) 500 nM Hg^{2+} ions. (C) Values of anisotropy of 6-FAM-T_{33} (50 nM) in 5 mM Tris−HCl solution (pH 7.4) plotted as a function of the Hg^{2+} concentration (0–120 μM). (D) Plot of the T_m value of Hg^{2+}−T_{33} as a function of the concentration of Hg^{2+} (0–1.2 μM). Other conditions were the same as those described in Figure 1.

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nuclear magnetic resonance spectroscopy.[14] The CD spectra of T_{33} (Figure S1, Supporting Information) indicated T_{33} (500 nM) changed from random-coil conformation to that of a folded structure upon increasing the Hg^{2+} concentration from 0 to 15 μM. To further support the formation of folded 6-FAM-T_{33} in the presence of Hg^{2+}, we recorded fluorescence polarization spectra of 6-FAM-T_{33} in the presence of various concentrations of Hg^{2+} (Figure 2C). Because of the relatively low sensitivity of fluorescence polarization spectroscopy, we used higher concentrations of 6-FAM-T_{33} and Hg^{2+} than those used in the CE-LIF experiments. The anisotropy of 6-FAM-T_{33} (50 nM) increased from 0.026 to 0.224 upon increasing the Hg^{2+} concentration from 0 to 120 μM, consistent with the putative changes in the structure of 6-FAM-T_{33}. The anisotropy in folded 6-FAM-T_{33} is higher than that in random-coil 6-FAM-T_{33} mainly because of the former's stiffer structure. We further conducted T_m measurements to support our reasoning. Upon increasing the temperature, the fluorescence intensity of TOTO-3·poly-T complexes (10 nM) decreased as a result of breaking in the T−Hg^{2+}−T bonding (Figure 2D). Upon increasing Hg^{2+} concentration, T_m increased and reached a plateau at the concentration of Hg^{2+} of 750 nM.

Effect of the Length of Poly-T and pH. Having observed that Hg^{2+} induced the formation of folded DNA structures, we suspected that the sensitivity of our analytical system would be dependent on the length of the DNA strand. We employed five poly-T ss-DNA samples of various lengths, T_7, T_{15}, T_{33}, T_{50}, and T_{80} (each 10 nM), to test our hypothesis. After plotting the values of $(I_{F0} - I_F)/I_{F0}$ for the TOTO-3·poly-T complexes against the concentration of Hg^{2+} (Figure S2, Supporting Information), we found that Hg^{2+} induced significant positive responses when the number of bases of poly-T was greater than 15. The degree of Hg^{2+}-induced fluorescence enhancement of the complex of TOTO-3 and T_7 was very small, mainly because of the difficulty of forming a folded structure from T_7. The values of $(I_{F0} - I_F)/I_{F0}$ for the complexes formed between TOTO-3 and T_{80}, T_{50}, and T_{33} in the presence of Hg^{2+} were similar: they all increased upon increasing the Hg^{2+} concentration and reached a plateau at a concentration of 500 nM.

We also explored the effect of pH of TOTO-3/T_{33} solution on sensing Hg^{2+}. As depicted in Figure S3 (Supporting Information), the values $(I_{F0} - I_F)/I_{F0}$ for TOTO-3·poly-T complexes were optimized at pH 7.4. The affinity of TOTO-3·DNA was reported optimized at pH 6−8, while Hg^{2+} binds directly to N3 of thymidine in place of the imino proton and bridges two thymidine residues to form the T−Hg^{2+}−T pair.[13] Thus, 5 mM Tris−HCl buffer (pH 7.4) was selected for the following studies.

Impact of Fluorophore. We investigated the effect of the TOTO-3 concentration on the sensing of 1 μM Hg^{2+} in the presence of 10 nM T_{33}. The values of $(I_{F0} - I_F)/I_{F0}$ for the complexes of TOTO-3, T_{33}, and Hg^{2+} increased upon increasing the concentration of TOTO-3 over the range of 0–20 nM (Figure 3A). At TOTO-3 concentrations greater than 20 nM, the sensitivity decreased as a result of a higher fluorescence background. We also investigated the use of some other DNA binding dyes (YOYO-3, TOTO-3, ethidium bromide, OliGreen, acridin orange, methyl blue, and the *N,N*-dimethyl-2,7-diazapyrenium dication; each 20 nM) and DNA nonbinding dyes (fluorescein and rhodamine B; each 20 nM) on the determination of Hg^{2+} using T_{33} (10 nM). Of these systems, the sensitivity for Hg^{2+} was highest when using the TOTO-3/T_{33} probe (Figure 3B). The values of $(I_{F0} - I_F)/I_{F0}$ for the best three dyes increased in the order TOTO-3 (K_b = ca. 10^9 M^{-1}) > YOYO-3 (K_b = ca. 10^7 M^{-1}) > ethidium bromide (K_b = ca. 10^6 M^{-1}), suggesting that the strength of binding of the dyes to T_{33} was an important factor determining the sensitivity. We note that the presence of Hg^{2+} induced slight increases in the fluorescence of mixtures of T_{33} and OliGreen, mainly because OliGreen is a dye that binds to single-stranded DNA. Fluorescein and rhodamine B did not bind to T_{33}; thus, their fluorescence intensities did not change significantly in the presence of Hg^{2+}.

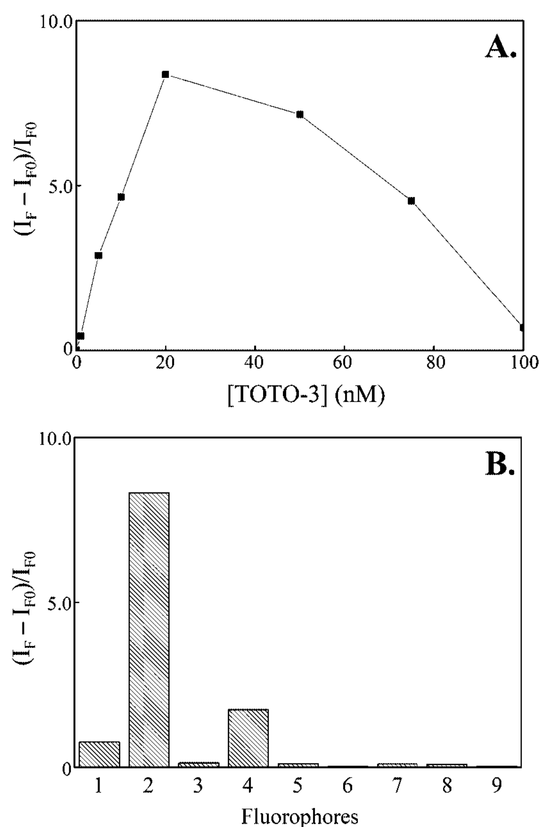

Figure 3. Values of relative fluorescence [$(I_F - I_{F0})/I_{F0}$] of 5 mM Tris−HCl solutions (pH 7.4) containing T_{33} (10 nM) and a selection of fluorophores. (A) TOTO-3 at various concentrations; (B) other fluorophores (20 nM). Key: (1) YOYO-3; (2) TOTO-3; (3) ethidium bromide; (4) OliGreen; (5) acridin orange; (6) methyl blue; (7) *N,N*-dimethyl-2,7-diazapyrenium dication; (8) fluorescein; (9) rhodamine B. Other conditions were the same as those described in Figure 1.

Sensitivity and Selectivity for Hg^{2+}. Under optimum conditions (20 nM TOTO-3, 10 nM T_{33}, and 5 mM Tris−HCl; pH 7.4), we observed that the value of $(I_F - I_{F0})/I_{F0}$ increased linearly (R^2 = 0.98) upon increasing the Hg^{2+} concentration over the range of 10–200 nM (Figure 4A). From a plot of $(I_{F0} - I_F)/(I_F - I_{Fs})$ versus the Hg^{2+} concentration, we calculated the formation constant of Hg^{2+} and T_{33} to be 5.8×10^6 M^{-1}, which is close to

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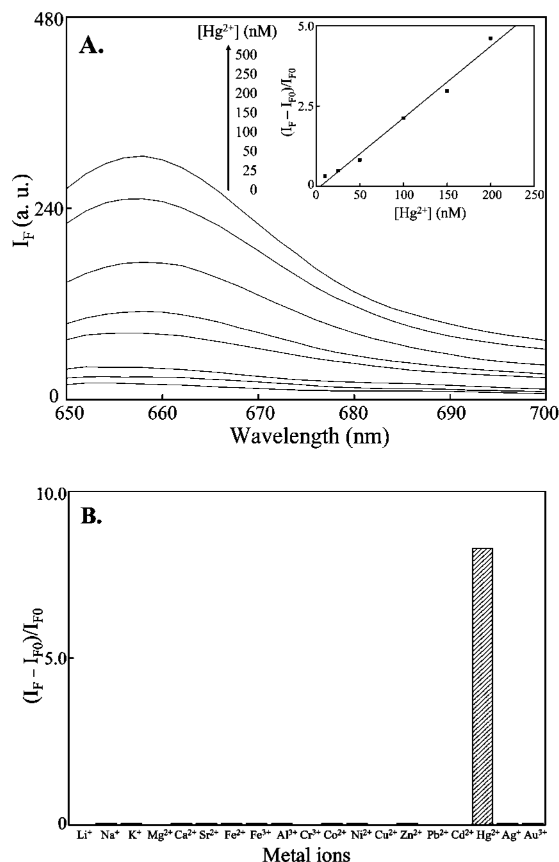

Figure 4. (A) Fluorescence responses of the T_{33}·TOTO-3 complexes after the addition of Hg^{2+} ions (0, 25, 50, 100, 150, 200, 250, 500 nM). Inset: linearity of the relative fluorescence [$(I_F - I_{F0})/I_{F0}$] at 660 nm with respect to the Hg^{2+} concentration over the range of 10–200 nM. (B) Relative fluorescence increases [$(I_F - I_{F0})/I_{F0}$] at 660 nm of 5 mM Tris−HCl solutions (pH 7.4) containing T_{33} and TOTO-3 upon the addition of 1.0 μM metal ions. The descriptors I_{F0} and I_F are the fluorescence intensities of the TOTO-3·T_{33} complexes in the absence and presence of Hg^{2+}, respectively. Other conditions were the same as those described in Figure 1.

the value we calculated from the CE-LIF data. The limit of detection (LOD) at a signal-to-noise ratio (S/N) of 3 was 3 nM (0.6 ppb), which is below the maximum level of mercury permitted by the U.S. EPA for drinking water. Thus, our present approach provides a sensitivity toward Hg^{2+} that is 1 order of magnitude better than that previously reported for a T−Hg^{2+}−T-mediated sensor.[7d]

Next, we investigated the selectivity of our new approach for Hg^{2+} over other metal ions (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Ag^+, and Au^{3+}; each 1.0 μM) under the optimum conditions. As indicated in Figure 4B, the TOTO-3/T_{33} probe was highly selective (265-fold or more) for Hg^{2+} over the other metal ions. We performed a series of competition experiments to test the practicality of our TOTO-3/T_{33} sensor for the selective detection of Hg^{2+}. The tolerance concentrations of various metal ions (within a relative error of ± 5%) for the sensing of Hg^{2+} (100 nM) using the TOTO-3/T_{33} probe were 1.0 μM for Au^{3+}, 5.0 μM for Ag^+, Cd^{2+}, and Pb^{2+}, and 10.0 μM for Cu^{2+}, Co^{2+}, Fe^{3+}, and Ni^{2+}. These results suggest that the metal ions we tested should not interfere with the determination of Hg^{2+} when applying our developed probe.

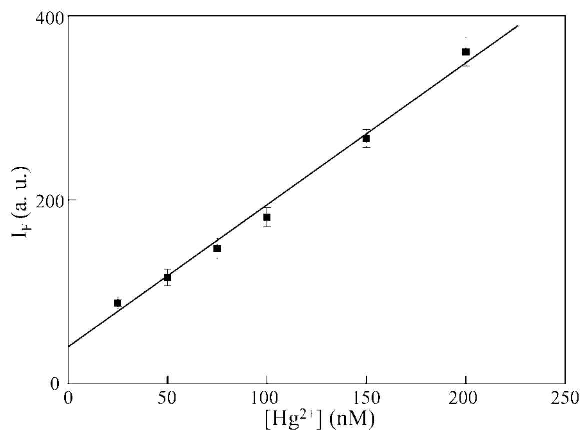

Figure 5. Linear response of the relative fluorescence [$(I_F - I_{F0})/I_{F0}$] of the T_{33}·TOTO-3 complexes after the standard addition of Hg^{2+} ions (25–200 nM) into diluted pond water. The dilution factor was 2. Other conditions were the same as those described in Figure 1.

Table 1. Concentrations of Hg^{2+} Determined in Three Different Types of Alkaline Manganese Batteries Using Two Different Methods

battery	TOTO/T_{33} probe, mean ± SD (mg/g; n = 5)	ICP-MS, mean ± SD (mg/g; n = 5)	F test between the two methods[a]
A	2.24 ± 0.07	2.19 ± 0.09	1.65
B	1.23 ± 0.06	1.26 ± 0.05	1.44
C	1.78 ± 0.10	1.82 ± 0.09	1.20

[a] F test value: 6.39 at 95% confidence.

Next, we investigated the effect that some chelating agents, including citrate, nitrilotriacetic acid, ethylene diamine tetracetic acid (EDTA), and imminodiacetic acid, have on the sensing capability of TOTO-3·T_{33} complexes toward Hg^{2+}. Only EDTA that forms strong complexes with Hg^{2+} has a significant effect on the sensing. When using TOTO-3·T_{33} complexes containing Hg^{2+} (200 nM), the fluorescence intensity decreased 71 and 85% in the presence of 200 nM and 2 μM EDTA, respectively (data not shown).[15] These results suggest that the TOTO-3·T_{33} sensor can be made renewable by using EDTA and conducting dialysis.

To evaluate the resistance of our sensor to endogenous nuclease degradation, we conducted sensing Hg^{2+} ions (0−1.2 μM) using TOTO-3·T_{33} (10 nM) in the presence of DNase I (100 nM). A linear plot (R^2 = 0.98) of the signal enhancement ratios $(I_{F0} - I_F)/I_{F0}$ of TOTO-3·T_{33} complexes against the concentration of Hg^{2+} (50–200 nM) is exhibited in Figure S4 (Supporting Information). This result indicated Hg-mediated folded structure resisted DNase I digestion.

Detection of Hg^{2+} in Real Samples. As indicated in Figure 5, the intensity of the fluorescence of the TOTO-3·T_{33} complexes increased upon increasing the spiked concentration of Hg^{2+} in pond water over the range of 25–200 nM (R^2 = 0.98). The recoveries of these measurements were valued at 97–108%. The LOD at an S/N ratio of 3 for Hg^{2+} in the presence of the complicated pond water matrix was 10 nM (2.0 ppb). We also applied our TOTO-3/T_{33} probe to the detection of Hg^{2+} in battery

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samples. Table 1 lists the concentrations of Hg^{2+} that we determined in three different types of batteries using both our developed probe and ICP-MS. On the basis of F-test, the results using our present approach are in good agreement with those obtained using ICP-MS. Note, however, that the sample preparation and analysis time when using the TOTO-3/T_{33} probe was less than 15 min; this assay provides the additional advantages of simplicity, low cost, and high throughput.

CONCLUSION

We have developed a homogeneous Hg^{2+} assay using TOTO-3 and T_{33}. Upon interaction with Hg^{2+}, T_{33} changes its conformation from a random coil to a folded structure, leading to an increase in the fluorescence intensity, electrophoretic mobility, and fluorescence anisotropy for the TOTO-3·T_{33} complex. This probe is highly sensitive and selective for Hg^{2+}. Although we have demonstrated the detection of Hg^{2+} ions only, we suspect that this probe strategy—using DNA samples of various lengths and sequences—will also be applicable to systems for the detection of metal ions such as Pb^{2+} and Cu^{2+}.

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SUPPORTING INFORMATION AVAILABLE

CD spectra of the T_{33} (500 nM) and TOTO-3 (1 μM) complexes after the addition of Hg^{2+} ions (0–20 μM) (Figure S1). Fluorescence responses of mixtures of TOTO-3 (20 nM) and poly-T (10 nM) after the addition of Hg^{2+} ions (Figure S2). Fluorescence responses of mixtures of TOTO-3 (20 nM) and T_{33} (10 nM) after the addition of Hg^{2+} ion (200 nM) in various pH value buffers (5 mM) (Figure S3). Relative fluorescence $[(I_F - I_{F0})/I_{F0}]$ of the T_{33}·TOTO-3 complexes in the presence of DNase I (100 nM) at 660 nm against Hg^{2+} concentration (0–1.2 μM) (Figure S4). This material is available free of charge via the Internet at http://pubs.acs.org.

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