Highly sensitive and selective detection of Hg^{2+} in aqueous solution with mercury-specific DNA and Sybr Green I†

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Sybr Green I efficiently discriminates mercury-specific DNA and mercury-specific DNA/Hg^{2+} complex, which provides a label-free, fast, fluorescence turn on assay for Hg^{2+} detection with high sensitivity and selectivity.

Mercury is a highly toxic element in ecosystems. Mercury contamination is widespread and arises from both nature and human activities. Mercury exposure can lead to a variety of adverse health effects such as damage to the brain, nervous system, immune system and many other organs.[1] Highly sensitive and selective Hg^{2+} sensors are in high demand for mercury pollution management and prevention. Fluorescence based Hg^{2+} detection has attracted a huge interest because of its simple operation, high sensitivity, and adaptability for in-field Hg^{2+} measurement.[2,3] Most fluorescence based Hg^{2+} detection utilizes small organic molecules. Other detection strategies based on nanoparticles,[4–9] conjugated polymers (CP),[10] DNAzymes,[11] foldamers,[12] proteins[13] and oligonucleotides[10,14] are also reported. Most detection strategies suffer from delayed response, cross-sensitivity toward other metal ions. Only a few sensors can detect Hg^{2+} in aqueous solutions with high sensitivity and selectivity.[15–18] As the toxic level for Hg^{2+} defined by the US Environmental Protection Agency (EPA) in drinkable water is below 10 nM, very few Hg^{2+} sensors can reach such sensitivity. There is an ever-growing challenge for developing highly sensitive and selective fluorescent Hg^{2+} sensors.

Very recently T–Hg^{2+}–T (T = thymine) chemistry has been highlighted in the development of Hg^{2+} sensors because T–T mismatch shows high selectivity to Hg^{2+} against many other metal ions.[19] A mercury specific DNA (MSD) has been elegantly designed for Hg^{2+} assays, which has a sequence of 5′-TTCTTTCTTCCCCTTGTTTGTT-3′. It forms a hairpin structure in the presence of Hg^{2+}, and presents a random coil form in the absence of Hg^{2+}. Three protocols have been reported using the MSD for Hg^{2+} detection. One is based on fluorescence resonance energy transfer (FRET) between fluorescein and dabcyl that has been labeled on both ends of a MSD.[14] In the absence of Hg^{2+}, fluorescein is separated from dabcyl and the system presents strong fluorescence, while in the presence of Hg^{2+}, the T–Hg^{2+}–T mediated hairpin formation causes dabcyl and fluorescein to come to close proximity for fluorescence quenching. The other two protocols are based on the absorption change of a cationic polythiophene or the surface plasmon resonance (SPR) absorption band of gold nanoparticles (AuNps) responding to Hg^{2+} induced conformational transition of the MSD probe, respectively.[8,10] All three protocols have shown high selectivity to Hg^{2+} against many other metal ions, however the sensitivity (40 nM for the first, 42 nM for the second, and 50 μM for the third) is still far above the toxic level of Hg^{2+} in drinkable water set by the EPA. Other T–Hg^{2+}–T chemistry based Hg^{2+} sensors are also known.[6,7] A colorimetric detection of Hg^{2+} based on two 21-mer DNA functionalized AuNps has shown a detection limit of 100 nM which requires thermal treatment at 45 °C.[6] Introducing an appropriate complementary linker and more T–T mismatches to the DNA probes immobilized on the AuNps, a detection sensitivity of 3 μM is obtained at room temperature.[7] A simplified colorimetric method was subsequently reported to show a detection limit of 250 nM based on Hg^{2+}–DNA complexation induced aggregation of naked AuNps.[9] The highest sensitivity for DNA based Hg^{2+} detection is reported to be 2.4 nM, which operates on a T–Hg^{2+}–T modulated DNAzyme through allosteric interaction.[11] This strategy is complicated and requires a dual labeled quencher system. In this communication, we use the same MSD as a probe and a DNA staining dye Sybr Green I (SG) as a signal reporter for Hg^{2+} detection. Our sensor strategy represents the simplest T–Hg^{2+}–T based fluorescent Hg^{2+} detection protocol with high sensitivity. The detection can be completed in less than 5 min.

SG is by far the most sensitive reagent for staining double-stranded DNA (dsDNA) and it has been successfully used in DNA qualification and quantification technologies such as gel electrophoresis and real-time PCR.[20,21] SG shows weak fluorescence upon binding to ssDNA, and there is ~11-fold fluorescence increase upon binding to dsDNA to yield a quantum yield of ~0.8.[22] Such discrimination arises from the different nature of SG upon interaction with ssDNA and dsDNA. SG interacts with ssDNA through electrostatic interactions, while SG binds to dsDNA through both intercalation and minor groove binding.[22] As a consequence, SG binds to free MSD and the MSD hairpin in different ways, which can induce a distinguishable fluorescence increase in response to the target-induced conformational transition of MSD. The schematic description of the detection protocol is shown in Fig. 1.

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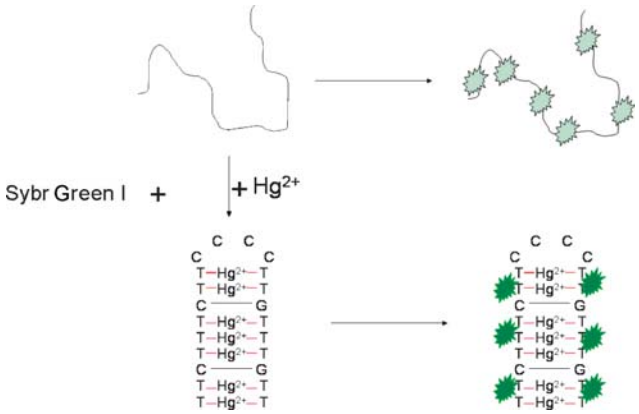

Fig. 1 Schematic description of the fluorescent Hg^{2+} sensing mechanism.

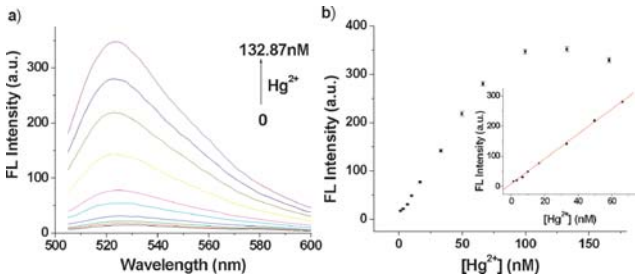

Fig. 2 (a) Fluorescence spectra of SG-MSD in the absence and presence of Hg^{2+}. (b) Fluorescence intensity of SG-MSD *vs.* $[Hg^{2+}]$. [MSD] = 1.58×10^{-8} M and [SG] = 8.14×10^{-8} M. A buffer of 10 mM 3-morpholinopropanesulfonic acid (MOPS), 0.1 M $NaNO_3$, pH 7.5 was used.

We first optimized the dye/base pair ratio (dbpr) for the MSD based detection. The dbpr was chosen to be 0.57 to achieve the highest ratio of the fluorescence intensity of SG–Hg^{2+}–MSD to that of SG–MSD (Fig. S1a in ESI†), at which the concentration of MSD and SG was 1.58×10^{-8} and 8.14×10^{-8} M, respectively. Under these conditions, the incubation time was optimized to 2 min for SG to bind to the Hg^{2+}–MSD hairpin to achieve the highest SG emission intensity (Fig. S1b in ESI†). As shown in Fig. 2, in the absence of Hg^{2+}, the solution showed a very weak fluorescence (14.5 a.u.). In the presence of Hg^{2+}, the fluorescence increased to 351.7 a.u. at $[Hg^{2+}]$ = 132.87 nM. The discrimination between SG–MSD and SG–Hg^{2+}–MSD was also reflected in the changes in the fluorescence emission maxima. In the absence of Hg^{2+}, the emission maximum of the SG–MSD solution was at 528 nm. Addition of Hg^{2+} gradually blue-shifted the emission maximum to 523 nm, which indicated that MSD underwent a structural transition from ssDNA to a hairpin structure.[22] By measuring the fluorescence intensity at the emission maximum of SG–Hg^{2+}–MSD, a linear response of fluorescence intensity *vs.* $[Hg^{2+}]$ was observed in the range 0–66.43 nM; 1.33 nM was the experimentally estimated detection limit, which was more than 30-fold lower than for previously reported MSD based Hg^{2+} sensors.[10,14] It is also lower than the EPA limit of $[Hg^{2+}]$ in drinkable water. The performance of several major mercury sensors is summarized in Table 1, which demonstrates that our sensor has very high sensitivity.

Table 1 Summary of optical Hg^{2+} sensors operated in water

Method	Detection limit	Sensitivity	Working mode	Operation
SG-MSD	1.33 nM	High	Turn-on	Simple
FRET-MSD	40 nM[14]	Good	Turn-off	Simple
CP-MSD	42 nM[10]	Good	Turn-on	Simple
AuNps-MSD	50 μM[8]	Low	Colorimetric	Simple
AuNps and	100 nM[6]	Moderate	Colorimetric	Complex
T–Hg^{2+}–T	3 μM[7]			Complex
chemistry	250 nM[9]			Simple
DNAzyme	2.4 nM[11]	High	Turn-on	Complex
AuNps	5 nM[5]	High	Turn-off	Complex
	10 nM[4]	Good	Turn-on	
Chemosensors	0.1 μM[15]	Moderate	Turn-on	Simple
	0.5 μM[16]	Moderate		
	<10 nM[17]	High		
	50 nM[18]	Good		

We then evaluated the assay's selectivity to Hg^{2+}. The sensor was interrogated with a spectrum of metal ions, such as Co^{2+}, Ni^{2+}, Cd^{2+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+} ions (each 3.32 μM). The nonspecific ions did not obviously alter the shape or the intensity of the fluorescence spectra of SG-MSD (Fig. 3). Co-existence of other metal ions with Hg^{2+} in the sample also did not affect Hg^{2+} detection (Fig. S2 in ESI†). To further confirm the specificity of the MSD to Hg^{2+}, we employed an oligonucleotide of non-cognate sequence (DNAnc): 5′-AAGAAAGAAGGGGAACAAACAA-3′. The emission maxima of SG–DNAnc and SG–Hg^{2+}–DNAnc were observed at ~529 nm, which belonged to the characteristics of SG/ssDNA.[22] In addition, the fluorescence intensity did not show any obvious change in the absence and presence of Hg^{2+} (Table S1 in ESI†). These results clearly demonstrate that the SG–MSD based Hg^{2+} sensor is highly specific.

To understand the high sensitivity of the scheme shown in Fig. 1, we exploited circular dichroism (CD) measurement to study the interaction between the MSD and Hg^{2+}. CD spectroscopy is a conventional technique to study the conformational change of DNA, which could report the structural variations intrinsically and kinetically. A positive peak at 277 nm was observed for the free MSD in the CD spectrum (Fig. S3 in ESI†). With increased $[Hg^{2+}]$ in solution, a red-shift together with a decrease in intensity

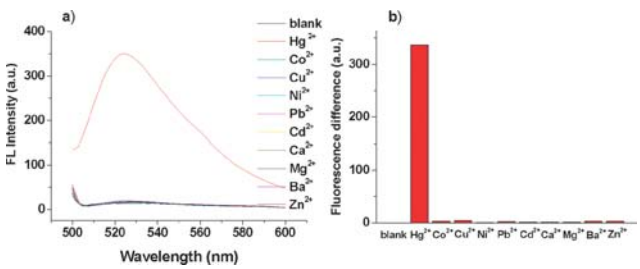

Fig. 3 (a) Fluorescence spectra of solutions containing MSD only (blank) or MSD in the presence of Hg^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, Zn^{2+} ions, respectively: [MSD] = 1.58×10^{-8} M; $[Hg^{2+}]$ = 132.87 nM; [non-specific ion] = 3.32 μM each. (b) The difference in fluorescence intensity between the blank and solutions containing different ions. Fluorescence difference = FL $Intensity_{(SG\text{-}metal\ ions\text{–}MSD)}$ − FL $Intensity_{(SG\text{–}MSD)}$.

was observed for the positive peak. Meanwhile, a negative peak appeared, which gradually increased in intensity and red shifted toward 270 nm. These results demonstrate that the MSD changes its structure from random coil to folded hairpin upon titration with Hg^{2+}. The ellipticity readings at 277 and 270 nm were plotted against $[Hg^{2+}]/7[MSD]$. A linear response was obtained for $[Hg^{2+}]/7[MSD] = 0$–1, which was followed by a plateau. This indicates that MSD kinetically accomplishes the hairpin structure at $[Hg^{2+}]/7[MSD] = 1$, where one equivalent of MSD requires 7 equivalents of Hg^{2+} to completely form the hairpin structure and all the thymines in the MSD participate in complexation. When the same experiments were done with Co^{2+} as a model for interfering ions, only a very small change appeared in either the shape or intensity of the CD spectra for $[Co^{2+}]/7[MSD] = 0$ and 1.17. This indicates that there is no structural change when MSD binds to Co^{2+}. These studies indicate that MSD is an excellent probe for Hg^{2+} because all the thymines in the sequence not only readily react with Hg^{2+}, but also shows a linear response upon formation of the hairpin structure. This is favorable for sensor design due to a clear and highly specific molecular recognition. The response of our sensor was interrogated with $[Hg^{2+}]/7[MSD]$ (Fig. S4 in ESI†). Results are consistent with the nature of MSD upon binding to Hg^{2+}, that is, the sensor is able to reflect the whole structural variation of MSD binding to Hg^{2+}, from $[Hg^{2+}]/7[MSD] = 0$ to 1, which agrees with the CD results.

In summary, we have developed a simple T–Hg^{2+}–T based fluorescent Hg^{2+} detection method using SG as a signal reporter. This detection strategy is simple and cost-effective which requires only label-free MSD. The detection could be done within 5 min with a detection limit of 1.33 nM, which is below the EPA limit for Hg^{2+} in drinkable water. The capability of SG to discriminate dsDNA and ssDNA should be generally applicable for other metal complexation DNA (M-DNA) based sensors, which can be employed to detect different metal ions and other analyte.[23–25]

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