Application of a DNA-based luminescence switch-on method for the detection of mercury(II) ions in water samples from Hong Kong

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Abstract
Mercury is a highly toxic environmental contaminant that damages the endocrine and central nervous systems. In view of the contamination of Hong Kong territorial waters with anthropogenic pollutants such as trace heavy metals, we have investigated the application of our recently developed DNA-based luminescence methodology for the rapid and sensitive detection of mercury(II) ions in real water samples. The assay was applied to water samples from Shing Mun River, Nam Sang Wai and Lamma Island sea water, representing natural river, wetland and sea water media, respectively. The results showed that the system could function effectively in real water samples under conditions of low turbidity and low metal ion concentrations. However, high turbidity and high metal ion concentrations increased the background signal and reduced the performance of this assay.

Keywords: mercury(II) ion detection, DNA based, luminescent probe, Hong Kong seawater

1. Introduction
Mercury is listed as a priority pollutant by many international agencies due to its persistence, bioaccumulation and toxicity in the environment. Mercury is extensively used in agriculture and industry for the manufacture of pesticides, fungicides, electrical goods, paper, batteries and other items, resulting in the release of large amounts of mercury into the environment. Another major source of atmospheric emission of mercury is the combustion of coal in coal-fired power plants (Gibb \textit{et al} 2000). Consequently, the biogeochemistry of mercury(II) ions (Hg\textsuperscript{2+}) in coastal and estuarial environments has received particular attention (Mason \textit{et al} 1996, Hines \textit{et al} 2000, Conaway \textit{et al} 2003, Schaefer \textit{et al} 2011). Bioaccumulation and trophic transfer processes in the environment lead to the concentration of mercury(II) in the tissues of high trophic level marine organisms, including edible marine species, posing risks to the local population.

Hong Kong contains a coal-fired station located on Lamma Island operated by the Hong Kong Electric Company (HEC), as well as several large industrial estates which are typically situated near important water systems such as Shing Mun River and Nam Sang Wai. In Hong Kong, mercury(II)
was detected in fish at Nam Sang Wai (Kong et al 2005) and Shing Mun River (Zhou and Wong 2000). Feathers of two Ardeid species in Mai Po Marshes next to Nam Sang Wai wetland were measured to contain up to 35.5 µM dry weight of mercury by inductively-coupled plasma mass spectrometry (ICP-MS) (Connell et al 2001). Furthermore, Perna viridis mussels collected at Lamma Island in Hong Kong waters were found to contain 0.3–0.51 µM dry weight of mercury using ICP-MS (Liu and Kueh 2005).

As Hong Kong suffers from mercury pollution, the sensitive monitoring of environmental contaminants is essential to safeguard citizens’ health. Mercury(II) can cause damage to the human brain, kidneys and lungs (Clifton 2007, Garrecht and Austin 2011), and can cause diseases such as acrodynia (Shandley and Austin 2011), Hunter–Russell syndrome (Kondo 2000) and Minamata disease (Davidson et al 2004). Therefore, the development of in-field detection methods for mercury(II) ions remains an important challenge (Davidson et al 2004, Holmes et al 2009).

Traditional detection methods for mercury(II) ions include atomic absorption spectroscopy (AAS) and ICP-MS. Despite their widespread use in laboratories and industry, their requirement for off-site laboratory and costly instrumentation limits their practical use for the in-field testing of mercury(II) ions. The target-induced structural switching of DNA (Lacroix et al 2011, Tran et al 2010, 2011, Mergny 2012) has been widely used in the design of DNA-based sensors (Willner and Zayats 2007, Li et al 2008, Willner et al 2008, Teller et al 2009, Freeman et al 2010, Leung et al 2010, Huang et al 2011, Ma et al 2011a, 2011b, Man et al 2011, Pu et al 2011, He et al 2012a, 2012b, 2012c, Leung et al 2012a, 2012b, Ma et al 2012a, 2012b, 2012c, Pelosoif et al 2012, Peng et al 2012). Our research group has previously developed a label-free DNA-based luminescent switch-on probe for mercury(II) ion detection (Chan et al 2009) without the need for expensive instrumentation or sample preparation. In the present study, we have applied this luminescence methodology for the in-field detection of mercury(II) ions in different environmental media including natural river, wetland and sea water samples close to industrial zones.

2. Experimental/materials and methods

2.1. Materials

The stock solution (1 mM) of platinum(II) complex was prepared in acetonitrile and was kept at −20°C in the dark before use. Further dilution to designated concentrations was made using Tris buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.2) or water samples. The mercury(II) chloride stock solution (1 mM) was stored in glass bottles with tight lids, and was further diluted to the designated concentrations by addition into the Tris buffer or water samples. All oligonucleotides were synthesized by Techdragon Inc. (Hong Kong, China). The sequence of the thymine-rich single-stranded oligonucleotide T33 is as follows: 5′-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3′.

2.2. Water sample collection

Water samples were collected from three locations in Hong Kong representing natural river, wetland and sea water environments, respectively. The sampling area in Shing Mun River was located next to Fo Tan Factory Estate, the sampling area in Nam Sang Wai wetland was located next to Yuen Long Industrial Estate, and the sampling area in Lamma Island was located next to the Lamma Power Station. To obtain the samples, 2 liters of water were collected at a depth of 1 m below the water surface at each sampling station using a stainless steel 45 mm diameter Vertical Bailer Water Sampler. The water samples collected were split into two 1 liter glass bottles which were immediately capped, chilled on ice and delivered to the laboratory, where they were stored at 4°C in the dark. Glassware was used throughout the experiments instead of polypropylene in order to prevent mercury(II) ions from binding to the polypropylene surface. All glassware was pre-cleaned according to a reported literature procedure (Polonini et al 2011). Glassware was ultrasonicated for 15 min, followed by rinsing with ethanol and double distilled water and then drying in a forced draft hot air oven at 100°C overnight.

2.3. Sample extraction

The water samples were thoroughly shaken each time before extraction to ensure that the determinant was uniformly distributed within the sample. Samples were extracted at 5 cm under the water level of bottle with an auto-pipette and filtered using a GD/XP 25 mm syringe filter with a 0.45 µm pore size. The blanks were prepared by adding the T33 (285.7 nM) and [Pt(C=N=N)(4−appt)]+ (9.74 µM) to 500 µl of filtered water sample.

2.4. Synthesis of the platinum(II) complex:

\[ \text{[Pt}(C=N=\text{N})(4−\text{appt})]+ \]

The synthesis of [Pt(C=N=\text{N})(4-appt)]+ (C=N=N = 6-phenyl-2,2'-bipyridine; 4-appt = 2-amino-4-phenylamino-6-(4-pyridyl)-1,3,5-triazine) was prepared as previously described without modification (Chan et al 2009).

2.5. Instrumental analysis

2.5.1. ICP-MS

The concentrations of mercury(II) ions in untreated water samples were analyzed using inductively-coupled plasma mass spectrometry (ICP-MS) from a commercial testing laboratory (Hong Kong Standards and Testing Centre). Concentrations of mercury(II) ions in the sea water samples were measured by the Hong Kong Standards and Testing Centre (detection limit = 500 nM), while the samples from natural river and wetland were measured by the CMA Testing and Certification Laboratories (detection limit = 50 nM). Quality control for ICP-MS determination was performed by spike-and-recovery analysis.
2.5.2. Emission measurement. Solutions of the platinum(II) complex (9.74 µM) with T33 oligonucleotide (285.7 nM) in Tris buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.2) or water samples were added to a cuvette. Aliquots of a millimolar Hg2+ stock solution (0–15 µM) were then spiked to 500 µl of Tris buffer or water samples. After equilibration at room temperature for 10 min, luminescence emission spectra were recorded on a PTI QM-4 spectrofluorometer at 25 °C. The emission intensity at 400–800 nm was monitored after excitation of the sample at 360 nm.

2.5.3. Turbidity test. Thermo Orion AQ3010 AQUAfast IV Turbidimeter Kit was used to determine the turbidity of water samples after filtration with GD/XP 25 mm syringe filter. Standards, buffers and samples were measured in EPA180.1 mode and the measuring unit was in NTU.

2.6. Data analysis

Intensity fold-change of each concentration of [Hg2+] was calculated by dividing its luminescence emission intensity at 537 nm by the emission intensity of [Hg2+] = 0 nM.

3. Results and discussion

3.1. Characterization of mercury(II) ion detection system in buffer solution

It has been previously reported that single-stranded thymine-rich DNA sequences can be induced into a hairpin conformation by mercury(II) ions (Miyake et al 2006) though formation of T-Hg2+-T mismatches (Ono and Togashi 2004, Miyake et al 2006, Tanaka et al 2006, Torigoe et al 2010, Ono et al 2011, Uchiyama et al 2012). The mechanism of our previously developed luminescent assay for mercury(II) ions is depicted in figure 1. The luminescent cyclometalated platinum(II) complex [Pt(C=N=N)(4-appt)]+ (1, C=N=N = 6-phenyl-2,20-bipyridine; 4-appt = 2-amino-4-phenylamino-6-(4-pyridyl)-1,3,5-triazine; figure 2) is weakly emissive in aqueous buffer solution due to complex-solvent interactions that result in the non-radiative decay of its excited state. In the absence of mercury(II) ions, complex 1 is weakly emissive due to its weak interaction with the single-stranded oligonucleotide T33. However, in the presence of the mercury(II) ions, complex 1 intercalates into the double-stranded hairpin conformation induced by mercury(II) ions. This protects the metal complex from the aqueous buffer environment and suppressed non-radiative decay, thus enhancing 3MLCT luminescence. (Chan et al 2009). By measuring the luminescence of the system, the assay was presently determined to achieve a detection limit of 20 nM for mercury(II) ions in buffer solution, with a maximum intensity fold-change of 5.19 reached at a saturation limit of above 15 µM (table 1).

3.2. Characterization of the mercury(II) ion detection system in water samples.

In order to investigate the accuracy and reliability of the luminescent system in different media, the probe was applied to water samples from natural river, wetland and sea water samples. Quality control for the probe was initially planned by comparison with results obtained using inductively-coupled plasma mass spectroscopy (ICP-MS). However, ICP-MS analysis of the three water samples showed that they contained insignificant concentrations of mercury(II) ions. Therefore, the water samples were spiked with mercury(II) ions for the purpose of the experiments. The precision of the system was
evaluated by the use of triplicate measurements of the water samples.

A calibration curve for the luminescence intensity of the system at a range of mercury(II) concentrations in buffer solution was first constructed (figure 3). The relationship between intensity fold-change and mercury(II) concentration in the buffered solution was found to be linear in the range of 0.5–15 µM ($R^2 = 0.9946$). The real water samples were then spiked with various concentrations of mercury(II) ions, and the resulting luminescence intensity was compared with the calibration curve to determine the estimated mercury(II) concentration. Spike recovery analysis was performed in triplicate to evaluate the performance of the detection system in water samples compared to buffer solution.

3.2.1. Characterization of the mercury(II) ion detection system in low turbidity and low metal ion water samples.

In natural river samples, the turbidity level was determined to be about 28 NTU, compared to 0.77 NTU for the buffer solution. The water sample also contained a relatively low concentration of metal ions compared to that of sea water (Solà and Prat 2006). The luminescence intensity of the system was recorded in triplicate at various concentrations of spiked mercury(II) ions in the natural river water samples (figure 4). The results showed a linear dynamic range from 0–12 µM ($R^2 = 0.9869$) for Hg$^{2+}$ ions with a maximum emission intensity fold-change of about 4.3, which is comparable to the performance of the assay in buffer solution. At higher concentrations of Hg$^{2+}$ ions, the luminescence emission of the system was observed to plateau, presumably due to the saturation of Hg$^{2+}$ ion binding sites in the $T_{33}$ oligonucleotide. This phenomenon can be found in both aqueous buffer and real water sample, while the luminescence emission of the system in aqueous buffer requires higher concentrations of Hg$^{2+}$ ions to attain the plateau compared to that in the real water sample. The system exhibited modest percentage recovery values for spiked mercury(II) ions in the natural river water samples as compared to the buffer solution (table 2). The detection limit for mercury(II) ions in the natural river sample was determined to be 100 nM. Significantly, this detection limit for mercury(II) ions is comparable to those offered by testing agencies in Hong Kong (50–500 nM). The higher detection limit for mercury(II) ions in the river sample compared to that for the buffer solution (20 nM) could be due to the shielding of the luminescence signal at low mercury(II) ion concentrations by the moderate turbidity present in the real water sample. Furthermore, the selectivity of our approach for Hg$^{2+}$ ions was evaluated by investigating the luminescent response of the system to 100-fold higher concentrations of nine other common metal ions, including Pb$^{2+}$, Mg$^{2+}$, Ca$^{2+}$, Al$^{3+}$, Ti$^{3+}$, Sr$^{2+}$, Zn$^{2+}$, Na$^+$ and K$^+$ ions in natural river water sample. The results show that only Hg$^{2+}$ ions could significantly enhance the luminescence emission of the complex $I/T_{33}$ system (figure 5). The presence of Ca$^{2+}$ or Al$^{3+}$ ions changed the luminescence response of the system by around 6–10%. These results indicate that the system displays excellent selectivity for Hg$^{2+}$ ions over the nine other metal ions tested in the natural river sample.

3.2.2. Characterization of the mercury(II) ion detection in high turbidity.

The wetland water samples had an average turbidity measurement of 269.3 NTU (table 3), which was about 350 times higher than that of aqueous buffer solution (0.77 NTU). The high turbidity of the sample, which is likely caused by suspended particles such as organic matters and soil (Blavet et al. 2009), resulted in a significant background emission signal (figure 6). The maximum fold-change of the
Table 3. Turbidity measurements of filtered water samples measured in EPA180.1 mode in NTU.

| Sampling locations     | Turbidity (in NTU) | pH  |
|------------------------|--------------------|-----|
|                        | 1st    | 2nd    | 3rd    | Average |     |
| Shing Mun River        | 26.9   | 25.6   | 31.8   | 28.1    | 7.94 |
| Nam Sang Wai           | 266    | 266    | 276    | 269.3   | 7.75 |
| Lamma Island           | 0.9    | 0.45   | 0.56   | 0.637   | 7.31 |
| Buffer                 | 0.86   | 0.78   | 0.69   | 0.77    | 7.2  |

Table 4. The detection limit, saturation point and luminescence intensity fold-change at saturation of the system for mercury(II) ions in samples obtained from three locations in Hong Kong.

| Sampling locations               | Detection limit (nM) | Saturation limit (µM) | Intensity fold-change |
|----------------------------------|----------------------|-----------------------|-----------------------|
| Shing Mun River (natural river)  | 100                  | 12                    | 4.28                  |
| Nam Shan Wai (wetland)           | 100                  | 4                     | 1.75                  |
| Lamma Island (sea water)         | 500                  | 8                     | 1.59                  |

Figure 5. Selectivity of the assay for Hg$^{2+}$ ions. The concentration of Hg$^{2+}$ ions was 4.0 µM and the concentrations of the other metal ions were 400 µM. Experimental conditions: $T_{33}$ (285.7 nM) and complex 1 (9.74 µM) in triplicate natural river samples.

Figure 6. Relationship between luminescence intensity fold-change of the system and mercury concentration (nM) in triplicate wetland water samples.

3.2.3. Characterization of the mercury(II) ion detection in high metal ion water samples. A high emission background signal was observed in the sea water sample. The four most abundant metal cations in sea water are sodium (469 000 µM), magnesium (53 000 µM), calcium (10 300 µM), and potassium (10 200 µM) (Holmes-Farley 2003). Our previous results have shown that the presence of micromolar metal ions could only induce a minute increase in the background signal of the detection system in the absence of mercury(II) ions. Although the sea water sample had a very low turbidity level similar to that of buffer solution, its detection limit for mercury(II) ions (500 nM) was the highest of all the water samples tested (figure 7). Only a 1.6-fold change in luminescence intensity was recorded at saturating concentrations of mercury(II) ions, and the recovery value of 1.8 was reached at a saturation limit of only 4 µM of mercury(II) ions. The detection limit of the system in wetland samples was determined to be 100 nM, which is comparable to that for the natural river samples (table 4). However, due to the turbidity of the sample matrix, the percentage recovery value for the spiked wetland sample was only about 50% at 4 µM of mercury(II) ions (data not shown).

4. Conclusion

In conclusion, we have successfully applied our recently developed DNA-based luminescent method for the detection of spiked mercury(II) ions in real environmental samples including natural river, wetland and sea water media. The results showed that the luminescent assay functioned effectively under conditions of low turbidity and low heavy metal ion concentrations in the natural river water sample. However, a higher background signal was observed due to the high turbidity of the wetland sample or high concentrations of natural metal ions in the sea water sample. The assay exhibited...
Figure 7. Relationship between luminescence intensity fold-change of the system and mercury concentration (nM) in triplicate sea water samples.

the best performance in the natural river sample with modest percentage recovery values and a detection limit of 100 nM.

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