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Detection of Hg^{2+} using molecular beacon-based fluorescent sensor with high sensitivity and tunable dynamic range

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ABSTRACT

A molecular beacon (MB) sensor was developed for highly sensitive and specific detection of Hg^{2+} ions with a tunable dynamic range. This method was based on the "turn-on" reaction of a hairpin DNA probe upon binding with mismatched target and Hg^{2+} ions through the formation of T-Hg^{2+}-T coordination. The conformational change of the MB caused a significant increase in fluorescence intensity, which could be used for Hg^{2+} sensing. The dynamic range of the sensor could be tuned by rationally controlling the number of T-T mismatches in between the MB loop and mismatched target DNA in the cases where detection of Hg^{2+} at different concentration ranges is required. With three T-T mismatches in the sequences, the sensor showed higher sensitivity with detection limit of 1.9 nM but narrower dynamic range. Further adding the number of T-T mismatches to seven, the sensor showed wider dynamic range but compromised sensitivity with detection limit of 44.2 nM. By mixing different mismatched targets in the assay, we found that both good sensitivity and wide dynamic range can be achieved. Adding small portion of T3 and T5 to T7 in the assay, the detection limit was significantly lower to 9.5 nM without sacrificing dynamic range. Based on the results, the mechanism of Hg^{2+} detection based on interaction between MB and mismatched targets was discussed. This sensor showed excellent selectivity toward Hg^{2+} ions in the presence of other metal ions. The proposed strategy was also able to detect Hg^{2+} in real water samples.

1. Introduction

Mercury pollution is a serious threat to the environment and human health. Mercury contamination originates from both natural and human activities such as volcanic eruption, mining and burning of fuels and waste [1–3]. Water-soluble mercuric ion (Hg^{2+}) is the most stable form of mercury which extensively distributed in atmosphere, soil and water [4]. It can accumulate in the ecological system through food chain and eventually enter human bodies [5,6]. Exposure even at low-level of Hg^{2+} can cause a number of severe health problems such as kidney damage, brain damage, and other chronic diseases [7–9]. Several conventional techniques have been developed for the detection of Hg^{2+} such as atomic absorption/emission spectroscopy [10–12], inductively coupled plasma mass spectrometry (ICP-MS) [13,14], cold vapour atomic fluorescence spectroscopy [15] and X-ray fluorescence spectrometry [16,17] Although these techniques provide sensitive and precise detections, they require time-consuming sample pretreatments, sophisticated equipment and are unsuitable for on-site detection. Considering the diverse sources of mercury and its high toxicity, highly sensitive and selective Hg^{2+} sensors are in high demand for understanding its distribution and pollution.

Recently, there is a growing interest of developing metal sensors based on functional nucleic acids [18–20]. It was previously reported by Ono and Togashi that Hg^{2+} can specifically bind to T-T base pairs and mediates the formation of stable T-Hg^{2+}-T complexes [21,22]. Since its discovery, T-T base pairs have been widely used to develop Hg^{2+} biosensors using different transduction mechanisms, such as fluorescence [21], colorimetry [23], and electrochemistry. [24]. Among these, fluorescent-based Hg^{2+} sensors have attracted much attention because of their high sensitivity, high selectivity, short response time and possibility for real-time applications. The first T-T mismatch-based fluorescent sensor was developed by Ono and Togashi in which a single stranded T-rich DNA was labelled with a fluorophore and a quencher at the two

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ends, respectively [21]. In the presence of Hg^{2+}, formation of T-Hg^{2+}-T base pairs brought the two ends in close proximity, resulting in fluorescence decrease due to fluorescence quenching effect. This sensor had detection limit of 40 nM. Subsequently, several "turn-off" sensors were also reported [25]. These "turn-off" sensors are sensitive and selective for Hg^{2+} detection, but they may give "false positive" results due to external quenching effect from external quenchers. Therefore, effort has been directed toward development of "turn-on" sensor. Lu and co-workers reported a turn-on sensor by introducing T-T mismatches in the stem of the uranium-specific DNAzyme with signal amplification by Hg^{2+} through allosteric interactions [26]. This sensor is highly sensitive with detection limit of 2.4 nM. However, it required the use of toxic uranium ions as cofactors. Several label-free sensors which utilized intercalating dyes, such as TOTO-3[27] and SYBR Green I [28,29], as well as conjugated polymers [30] in the design were also reported. Although these sensors showed high sensitivity, there is a huge controversy over the toxicity of some intercalation dyes. Besides, synthesis of organic dyes and polymers involve chemical reactions, and some of the chemicals may potentially be threats to the environment and human. Recently, there has been growing interest in using nanomaterials for the development of sensors, such as carbon nanotubes [31], graphene oxide [32] and nanoclusters [33]. However, the use of nanomaterials in turn will increase the cost of the sensors. Therefore, improving the design of oligonucleotide probe is more cost-effective than utilizing nanomaterials as quenchers.

Molecular beacons (MBs) are single stranded oligonucleotide probes in hairpin structure, with a fluorescent moiety and a quenching moiety attached to both ends. When the loop portion of the MB binds to its target molecule, it can emit fluorescence with enhancement as high as 200-fold under optimized conditions [34]. Recently, a MB-based fluorescent sensor was reported for Hg^{2+} detection with detection limit of 2.5 nM [35], but the detection range was quite narrow, 10–400 nM. Considering the samples from different locations contain different levels of Hg^{2+} ions, the development of a method with high sensitivity and wide detection range is desired to accommodate various detection criteria in different cases. Most of the reported strategies only show one detection range and it is often quite narrow, which make them not suitable for high concentration detection as the detection signal can easily reach saturation. Recently, a QCM-based sensor with a tunable detection range has been reported for detection of Hg^{2+} ions by altering the concentration of linker DNA [36]. Xu and co-workers also demonstrated a colorimetric sensor for Hg^{2+} detection with a tunable detection range based on DNA oligonucleotides and unmodified gold nanoparticles as sensing system [23]. However, the sensitivity of the sensors was compromised when the dynamic range was extended.

Since fluorescent sensors with a tunable detection range for Hg^{2+} detection have not been reported so far, it is of great interest to develop a fluorescent detection method with a convenient way of tuning the detection level while maintaining low detection limit. In this study, we aim to develop a MB fluorescent sensor with wide detection range and good sensitivity for Hg^{2+} detection. We also demonstrate a novel strategy for tuning the detection range and sensitivity of the sensor simply by controlling the number of T-T mismatches in the sensing system. This study opens a way to exploit molecular beacons for convenient quantification of Hg^{2+} ions involving different concentration ranges.

2. Experimental

2.1. Chemicals and materials

Tris(hydroxymethyl)aminomethane (Tris), mercury dichloride ($HgCl_2$) and other metal salts were purchased from Sigma–Aldrich.

Table 1
Names and sequences of the oligonucleotides. Bold T represents mismatched thymine residues and underline region represents the loop of the MB.

Name	Sequence
MB	5′-TAMRA/CCTCAGGCTGCGTAGTTGTGCTGATGCTGAGG/BHQ2-3′
T3	5′-CATC**T**GCAC**T**AC**TT**CGCAGC-3′ (3 T-T mismatches)
T5	5′-C**TT**C**T**GCAC**T**AC**TT**CGC**T**GC-3′ (5 T-T mismatches)
T7	5′-C**TT**C**T**GC**T**C**TT**CTTCGC**T**GC-3′ (7 T-T mismatches)

All chemicals were of analytical-reagent grade. Ultra-pure water at resistivity 18.2 MΩ cm was used in all preparations. All oligonucleotides used in the experiment were purchased from 1st Base Pte Ltd. (Singapore) and HPLC-purified. The sequences of the MB and three mismatched target DNAs (T3, T5 and T7) were designed as in Table 1.

10 mM Tris buffer containing 50 mM NaCl and 5 mM $MgCl_2$ at pH 8 was prepared as working buffer, except for experiments involving $MgCl_2$ salt optimization. DNA working solutions were prepared by dissolving the MB and mismatched target DNAs in the Tris buffer and further diluted in the buffer to 100 nM and 5 μM, respectively. All DNA solutions were stored at 4 °C and restored to room temperature before use.

2.2. Instrumentation

Fluorescence measurements were recorded on a Horiba Jobin Yvon FluoroMax-4 compact spectrofluorometer in a quartz cuvette with 1-cm lightpath length at room temperature. The excitation wavelength was set at 550 nm and the emission spectra were monitored.

2.3. Detection of Hg^{2+} ions

For the detection of Hg^{2+}, 600 μL of 100 nM MB was mixed with mismatched target DNA in equal mole and then a small amount of concentrated Hg^{2+} solution was added. After a gentle mixing, the mixture was allowed to incubate for 30 min. Subsequently, the fluorescence emission spectrum was recorded. The whole experiment was performed at room temperature.

For the sensitivity study, different concentrations of Hg^{2+} were added and the fluorescence emission spectra were monitored. For the selectivity study, the detection of 1000 nM other metal ions was done as the same as Hg^{2+} detection.

2.4. Hg^{2+} detection in lake water

A lake water sample was collected from Little Guilin Lake and filtered through 0.2 μm membrane prior to analysis. These samples were spiked with different concentrations of Hg^{2+} and added with concentrated buffer to make the final solution containing 10 mM Tris, 50 mM NaCl and 5 mM $MgCl_2$. The spiked samples were then added into the mixture of MB and mismatched target DNA and incubated for 30 min at room temperature. The fluorescence spectra were recorded as the same protocol as above.

3. Results and discussion

3.1. Sensing mechanism

The detection strategy of the sensing system is schematically shown in Fig. 1(A). A MB and three mismatched target DNAs with varying number of mismatched thymine residues in the sequences are designed. The essential idea of this method is to incorporate several T-T mismatched base pairs between the MB loop and the mismatched target DNA, such that the mismatched target DNA is