Molecular Sensors

Highly Selective Oligonucleotide-Based Sensor for Mercury(II) in Aqueous Solutions**

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Contamination with heavy metal ions may have severe effects on human health and the environment. Mercury contamination is widespread and arises from a variety of natural sources, such as oceanic and volcanic emissions,[1] as well as anthropogenic sources,[2] such as gold mining and the combustion of solid waste and fuels. Once introduced into the marine environment, bacteria convert inorganic mercury into methylmercury, which enters the food chain and accumulates in higher organisms, such as large edible fish.[3] Methylmercury is neurotoxic and has been implicated as a cause of mercury-pollution-related diseases.[4]

To increase our understanding of mercury pollution, efforts are being made to develop new mercury-sensing strategies that can detect mercury ions in the environment.[5] Such mercury sensors should display solubility in water and a high selectivity for mercury ions against a background of competing analytes. Small synthetic molecules offer one approach to such probes; many small-molecule sensors that rely on spectroscopic and colorimetric methods of detection have been prepared.[6] However, most of these molecules have limitations owing to interference from other metal ions, delayed response to mercury ions, and/or low water solubility. Only a few compounds have been reported to selectively detect Hg^{II} ions in aqueous solutions.[6n,o]

Herein, we report an oligodeoxyribonucleotide (ODN)-based sensing system that selectively detects Hg^{II} ions in aqueous solution. We recently observed the selective binding of Hg^{II} ions to thymine–thymine (T–T) base pairs in DNA duplexes.[7] As the binding of mercury by T–T pairs is strong and highly selective, duplexes that contain a T–T pair are thermally stabilized in the presence of Hg^{II} ions. In contrast, other heavy-metal ions, such as Cu^{II}, Ni^{II}, Pd^{II}, Co^{II}, Mn^{II}, Zn^{II}, Pb^{II}, Cd^{II}, Mg^{II}, Ca^{II}, Fe^{II}, Fe^{II}, and Ru^{II}, do not show any notable effects on duplex stability. Thus, a highly selective sensor for Hg^{II} ions that relies on the selective binding of Hg^{II} ions with a T–T pair could be envisaged.

The ODNs tested for the Hg^{II}-sensing system are shown in Figure 1. The sensor **D–ODN–F** consists of an ODN functionalized with a fluorophore (fluorescein, **F**) and a quencher moiety (dabcyl, **D**) at the 3′- and the 5′-termini, respectively.[8] Both the fluorescent and the quencher moieties are commercially available and are stable under the reaction conditions required for the chemical synthesis and deprotection of ODNs. The ODN sequence is divided into two parts: the thymine-rich mercury-binding sequence and the linker sequence. A mechanism for the detection of Hg^{II} ions by **D–ODN–F** is shown schematically in Figure 2. In the presence of Hg^{II} ions, mercury-mediated base pairs (T–Hg–T)

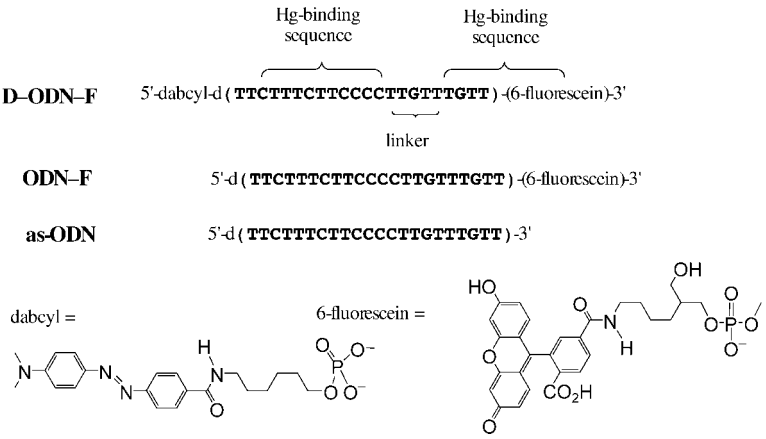

Figure 1. Structures of the ODN-based molecular sensors for mercury(II), **D–ODN–F**, **ODN–F**, and **as-ODN**.

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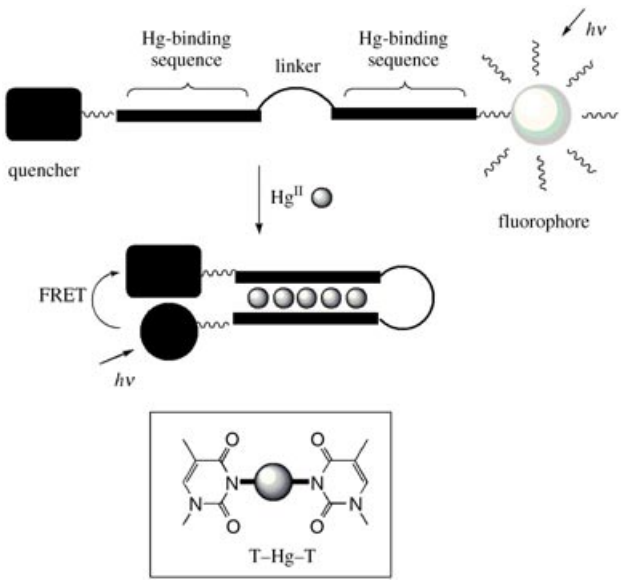

Figure 2. A schematic representation of the hairpin structure induced in **D–ODN–F** by Hg^{II} ion-mediated T–Hg–T pair formation, which results in the quenching of fluorescence from F.

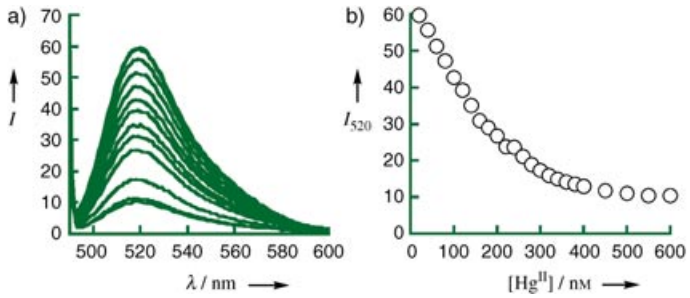

Figure 3. a) Fluorescence response of **D–ODN–F** (10 nM) upon addition of Hg^{II} ion (0, 20, 40, 60, 80, 100, 120, 140, 160, 200, 300, 400, 500, and 600 nM). The intensity of fluorescence emission decreased as the Hg^{II} ion concentration increased. b) Fluorescence emission intensity (520 nm) versus Hg^{II} concentration. A buffer solution of 3-(*N*-morpholino)propanesulfonic acid (10 mM, pH 7.0), NaCl (25 mM), $NaNO_3$ (500 mM), and ethylenediamine (0.1 mM) was used.

are formed between thymine residues from two Hg-binding sequences in the ODN to give rise to a hairpin structure. Both termini of the ODN are brought close to each other upon formation of the hairpin structure which leads to an enhanced fluorescence resonance energy transfer (FRET) process between the F and D moieties. Consequently, this results in significant quenching of the fluorescent emission relative to the random coil.

As shown in Figure 3a, the intensity of the fluorescence emission of **D–ODN–F** was sensitive to Hg^{II} ions and decreased as the concentration of Hg^{II} increased. A linear correlation between the emission intensity and the concentration of Hg^{II} ions was observed in the concentration range $40\ nM < [Hg^{II}] < 100\ nM$ (Figure 3b; detection limit = 40 nM, three times the standard deviation of the blank solution). The variation of the fluorescence intensity was not linear for Hg^{II} concentrations lower than 40 nM probably owing to the requirement of several Hg^{II} ions bound to one sensor molecule for formation of the hairpin structure. The sensitivity of this oligonucleotide-based sensor is greater than those of the previously reported small molecular sensors for Hg^{II}.[6n,o]

As well as high sensitivity, the sensing system requires high selectivity towards the Hg^{II} ion. Fluorescence spectra of **D–ODN–F** recorded in the absence and presence of heavy-metal ions are shown in Figure 4a. The addition of a mixture of other metal ions (Ca^{II} and Mg^{II} (both 1 mM), and Cu^{II}, Fe^{II}, Cd^{II}, Pb^{II}, Zn^{II}, Ni^{II}, Mn^{II}, and Co^{II} (each 1 μM)) did not alter the shape or the intensity of the fluorescence spectra (Figure 4a, +Mix). In contrast, the fluorescence emission spectrum was sensitive to the presence of Hg^{II} ions and its intensity decreased as the Hg^{II} concentration increased. The fluorescence spectra of a control oligodeoxyribonucleotide, **ODN–F**, which did not carry the quencher moiety D, were identical in both the absence and the presence of Hg^{II} ions (Figure 4b).

The reliability of the Hg^{II} sensing can be corroborated by reference to a control, which, in this case, uses an antisensor oligodeoxyribonucleotide (**as-ODN** in Figure 1) that has the same sequence as **D–ODN–F**, but does not carry the **F** and **D** residues. In the presence of excess **as-ODN**, the fluorescence spectrum of **D–ODN–F** changed only slightly upon addition

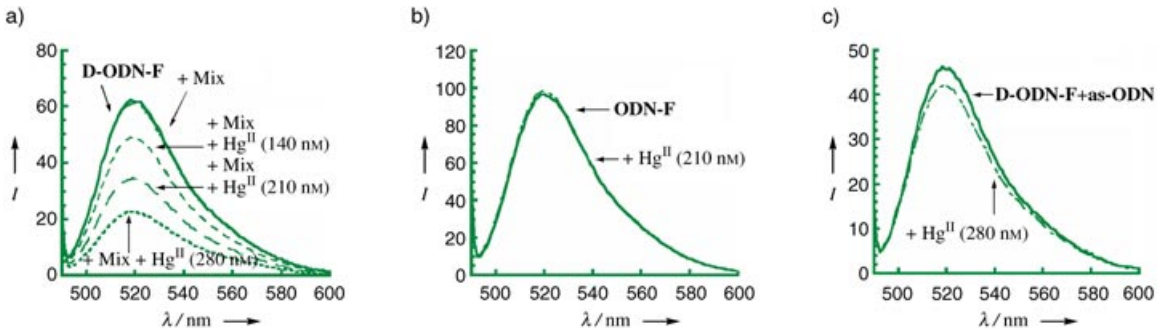

Figure 4. Fluorescence spectra. a) Solutions containing **D–ODN–F** (10 nM), a mixture of heavy metal ions (Mix; $CaCl_2$ and $MgCl_2$ (both 1 mM), and $CuCl_2$, $FeCl_2$, $CdCl_2$, $PbCl_2$, $ZnCl_2$, $NiCl_2$, $MnCl_2$, and $CoCl_2$ (each 1 μM)), and an appropriate concentration of $Hg(ClO_4)_2$. b) Solutions containing **ODN–F** (10 nM) in the absence or presence of $Hg(ClO_4)_2$. c) A solution containing **D–ODN–F** (10 nM) and **as-ODN** (1 μM) in the absence or presence of $Hg(ClO_4)_2$. The same buffer as described in Figure 3 was used.

of Hg^{II}, as most of the Hg^{II} ions were taken up by **as-ODN** and the Hg^{II} ion concentration decreased substantially (Figure 4c). In the analysis of environmental samples, the fluorescence of sensors can be quenched through the direct interaction of the fluorophores with unexpected substances in the sample media. In the present system, this unexpected quenching can be distinguished from Hg^{II}-derived quenching by using **as-ODN** as a control. When the fluorescence of **D–ODN–F** is quenched in the absence of **as-ODN**, but maintained in the presence of **as-ODN**, the sample contains Hg^{II} ions. Conversely, when the fluorescence of **D–ODN–F** is quenched in the presence *and* the absence of **as-ODN**, the quenching is most likely caused by unexpected contaminants in the sample and the presence of Hg^{II} in the sample is uncertain.

Consequently, our ODN-based Hg^{II}-sensing system has several advantages. First, the sensitivity of the system to Hg^{II} ions is higher than those of existing small molecular sensors. Moreover, the ODN-based sensing system can selectively detect Hg^{II} ions in a solution that contains excess amounts of other heavy metal ions. The reliability of Hg^{II} sensing can be corroborated by performing control experiments using a properly designed antisensor oligodeoxyribonucleotide. Furthermore, the sensitivity of ODN-based sensors can be regulated easily by variation of the sequences of metal-ion-binding sites and linkers.[9] Another advantage of the system is that fluorescence emissions at various wavelengths can be used to indicate different metal ions through variation of the donor–acceptor combinations for FRET.[10]

We hope that our findings will help to improve the direct detection of Hg^{II} ions in the environment and in the presence of excess mono- and divalent ions and other contaminants. Furthermore, our results demonstrate the utility of the oligonucleotide-based molecular design strategy in the development of molecular sensors.[7,11,12]

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[9] Besides **D–ODN–F**, the ODN-based Hg sensors **D–(T_{21})–F** and **D–($T_4C_4T_4$)–F** were also synthesized. As with **D–ODN–F**, the fluorescence emission spectra of **D–(T_{21})–F** and **D–($T_4C_4T_4$)–F** were quenched by Hg^{II} ions. However, the sensitivities of these sensors for the Hg^{II} concentration varied (see Supporting Information).

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