

Enhanced response of an oligonucleotide-based biosensor to environmental mercury

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Introduction

One environmental pollutant of particular relevance to the coal-generated power industry is mercury. Power plants in the U.S., led by Texas, Ohio, Pennsylvania, Indiana, and Alabama, collectively emitted over 90,000 pounds of mercury into the air in 2003. Calls for increased mercury monitoring activities have come from many groups concerned with environmental contamination and mercury bioconcentration in fish. Additionally, the benefits of improvements in the reduction of mercury emissions from existing power plants cannot be seriously evaluated without extensive monitoring of the environment. Low *in situ* mercury concentrations and the expense of traditional laboratory analyses currently limit such routine and effective monitoring.

Microbial biosensors sensitive to mercury have been developed that quantitatively produce light in response to the amount of mercury (II) entering the cells. However, these sensors are typically difficult to prepare, can have long lag times between initial exposure and subsequent light emission, and are difficult to use in the field. Whole cell biosensors using living bacteria also require attention to the growth requirements of the cells, as well as complications brought on by the presence of other toxic compounds in addition to mercury.

In this paper, I investigated the use of a novel "molecular beacon" sensor for mercury (II), recently reported by Ono and Togashi (*Angew. Chem. Int. Ed.* 2004, 43:4300-4302.). This method is based on the observed selective binding of mercury ions to thymine-thymine (T-T) base pairs in DNA duplexes. An oligonucleotide sequence is used that changes its conformation upon binding with mercury ions, causing a fluorophore at one end of the oligonucleotide sequence to come in proximity with a quencher molecule attached to the other end. Enhanced fluorescence resonance energy transfer (FRET) results in a decrease in the intensity of the fluorescence spectrum, which is correlated with the mercury concentration. The fluorescence spectrum generated by this sensor can be analyzed using a field spectrofluorometer, and the analytical approach may be useful in the future monitoring of environmental mercury concentrations.

Molecular Beacon

5'-6-FAM (CTTCTTCTTCCCCCTGTTTGT)-DAB-3'

The molecular beacon used previously consisted of two mercury-binding oligonucleotide sequences (underlined), connected by a linker sequence (CCCC), and terminated by the fluorophore and quencher moieties, fluorescein (6-FAM) and dabcyl (DAB), respectively. In this configuration, the fluorophore exhibits its maximum UV fluorescence at 520 nm.

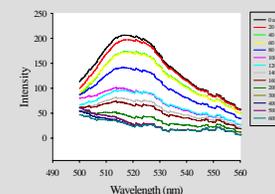
CTTCTTCTT-DAB-3'

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C
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CTTCTTCTT-6-FAM-5'

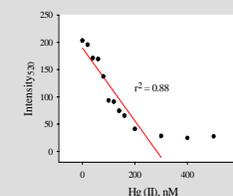
In the presence of Hg (II) ions, the two oligonucleotide sequences are brought together by the formation of T-Hg-T pairs, creating a hairpin structure. This brings the fluorophore and quencher molecules in closer proximity to one another and reduces the intensity of the fluorescence of the beacon. The quantitative reduction in fluorescence intensity is the basis for mercury detection using this approach.

Results

Response of biosensor to Hg (II)

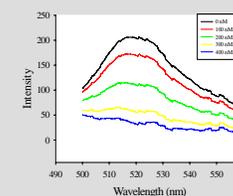


Fluorescence Emission Intensity (520 nm) vs Hg(II) concentration

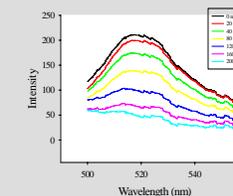


A linear decrease in biosensor fluorescence intensity was observed over the concentration range 20 – 200 nM Hg (II). Concentrations above 200 nM Hg (II) presumably saturated the Hg-binding sites on the available oligonucleotide.

Hg-spiked Metal Mix



Hg-spiked Youghiogheny River Water



The molecular beacon sensor detected increasing amounts of Hg (II) in a buffer containing 1 mM concentrations of Ca and Mg, and a mixture of heavy metals (1 uM each of Cu, Fe, Cd, Pb, Zn, Ni, Mn, and Co). Mercury dilutions prepared in natural river water likewise showed a biosensor response pattern similar to that generated by mercury in deionized water.

Materials & Methods

- The molecular beacon and analogs were synthesized by Integrated DNA Technologies, Coralville, IA.
- Sample dilutions were made in a buffer solution of MOPS (10 mM, pH 7.0), NaNO₃ (500 mM) and ethylenediamine (0.1 mM).
- Samples were analyzed using a Curie Fluorescence System (Ocean Optics Inc., Dunedin, FL), a high-sensitivity cuvette spectrofluorometer.
- Surface water from the Youghiogheny River was collected, spiked with mercuric chloride, and analyzed within two hours of collection.



Conclusions

- The molecular beacon responded well to aqueous Hg (II) concentrations between 20 – 200 nM, and appears to be relatively insensitive to the presence of additional ions;
- The analytical method is rapid and relatively inexpensive;
- The concentration of Hg (II) detected by this system is an order of magnitude greater than that typically found in environmental samples (pM). Methods to increase the sensitivity of this approach are currently under investigation.