

Screening Mercury Levels in Fish with a Selective Fluorescent Chemosensor

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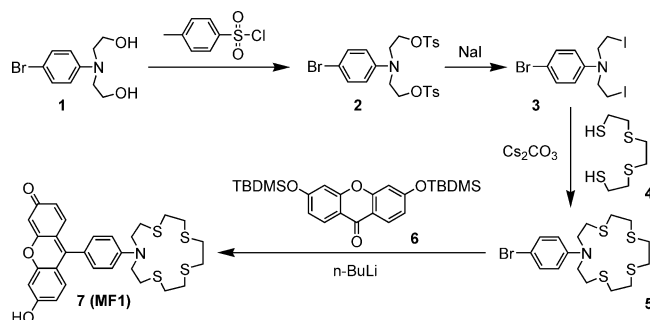
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Mercury is a dangerous and widespread global pollutant.¹ The long atmospheric residence time of Hg⁰ vapor and its oxidation to soluble inorganic Hg²⁺ provides a pathway for contaminating vast amounts of water and soil.² A significant problem stemming from this ecological oxidation chemistry is that bacteria living in the sediments of aqueous environments transform inorganic Hg²⁺ into methylmercury, a potent neurotoxin that concentrates through the food chain in the tissues of fish and marine mammals. Subsequent ingestion of methylmercury by humans from seafood and other dietary and environmental sources is connected to serious sensory, motor, and cognitive disorders.³

Concerns over toxic exposure to mercury provide motivation to explore new methods for monitoring aqueous Hg²⁺ from biological and environmental samples. Current techniques for mercury screening, including atomic absorption/emission spectroscopy⁴ and inductively coupled plasma mass spectrometry,⁵ often require expensive and sophisticated instrumentation and/or sample preparation. Fluorescence detection with Hg²⁺-responsive chemosensors offers a promising approach for simple and rapid tracking of mercury ions for biological, toxicological, and environmental monitoring. An important practical challenge to achieving this goal is devising water-soluble fluorescent dyes that report Hg²⁺ selectively over competing metal ion contaminants. Several types of small molecules,^{6–18} DNAzymes,¹⁹ and protein²⁰ or oligonucleotide²¹ platforms have been examined for fluorescence Hg²⁺ detection. However, none of these probes have been utilized successfully for sensing Hg²⁺ in natural samples, as available Hg²⁺-responsive fluorophores are often limited by nonspecific interference from Cu²⁺, Pb²⁺, and other competing metal ions, incompatibility with aqueous media, and/or delayed or irreversible Hg²⁺ response. We now present the synthesis and properties of Mercuryfluor-1 (MF1), a new water-soluble fluorescent chemosensor for screening mercury levels in fish. This reagent features excellent selectivity for Hg²⁺ over competing analytes, including common metal ion contaminants Cu²⁺ and Pb²⁺, and the largest fluorescence enhancement to date for sensing Hg²⁺ in water (>170-fold). Experiments with fish collected from field studies show that MF1 is capable of reliably detecting mercury levels in fish over a range of 0.1 to 8 ppm, establishing the utility of this probe for assaying fish for safe human consumption according to guidelines suggested by the U.S. EPA.¹

MF1 combines a fluorescein reporter²² having desirable optical properties and water solubility with a thioether-rich crown receptor to favor selective and stable binding of soft Hg²⁺ in water.²³ Scheme 1 outlines the synthesis of MF1. Reaction of 4-bromo-*N,N*-bis(2-hydroxyethyl)aniline **1** and *p*-toluenesulfonyl chloride affords ditosylate **2** in 71% yield. Conversion of **2** with sodium iodide proceeds smoothly to generate the corresponding diiodo compound **3** in 75% yield. Cyclization of diiodo **3** and 3,6-dithiaoctane-1,8-dithiol **4** with Cs₂CO₃ under high-dilution conditions produces the azathiacrown receptor **5** in low yield (9%). Lithium-mediated coupling²⁴ of macrocycle **5** and 3,6-bis[(1,1-dimethylethyl)dimethylsilyl]oxy]-9*H*-xanthen-9-one **6** furnishes MF1 **7** in 37% yield.

Scheme 1. Synthesis of Mercuryfluor-1 (MF1)

Spectroscopic measurements under simulated physiological conditions (20 mM HEPES buffer, pH 7) reveal that the optical properties of MF1 are dominated by the fluorescein chromophore. In the absence of Hg²⁺, MF1 has a visible absorption band centered at 485 nm ($\epsilon = 2.0 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) with a corresponding emission maximum at 514 nm. MF1 is virtually nonfluorescent in its apo state ($\Phi < 0.001$), indicative of efficient photoinduced electron transfer (PET) quenching of the fluorophore by the azathiacrown receptor. Upon addition of Hg²⁺, the fluorescence intensity of MF1 increases by over 170-fold ($\Phi = 0.16$, Figure 1A). This dramatic turn-on response is accompanied by red shifts in both excitation (495 nm, $\epsilon = 4.9 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) and emission (517 nm) maxima. Binding analysis using the method of continuous variations establishes that a 1:1 Hg²⁺:MF1 complex is responsible for the observed fluorescence enhancement, and the EC₅₀ for 1 μM MF1 is 700 nM. Using 1% of the total dynamic range as a cutoff (1.7-fold fluorescence increase), a 60 nM detection limit for aqueous Hg²⁺ is obtained for MF1.

MF1 is highly selective for Hg²⁺ over competing metal ion analytes in aqueous solution. Figure 1B depicts the fluorescence responses of a 1 μM solution of MF1 to the presence of various environmentally relevant metal ions. The emission profiles of MF1 or its Hg²⁺-bound form are unperturbed by millimolar concentrations of Li⁺, Na⁺, K⁺, Mg²⁺, and Ca²⁺, indicating excellent selectivity over these alkali and alkaline earth cations. MF1 is also selective for Hg²⁺ over first-row transition metal ions Mn²⁺, Fe²⁺, Co²⁺, Ni²⁺, and Cu²⁺ at 67-fold excess. The observed selectivity of MF1 for Hg²⁺ over Cu²⁺ is notable and reveals a greater affinity of the NS4 macrocycle for the former.²³ In addition, MF1 is also selective for Hg²⁺ over group 12 ions Zn²⁺ and Cd²⁺, as well as the common heavy metal ion pollutant Pb²⁺.

With a firm understanding of the spectroscopic properties and Hg²⁺ responses of MF1 in hand, the fluorescein-based probe was applied to provide a rapid screen for total mercury content in fish. Assays employed fish collected from field studies whose mercury content was verified by atomic absorption spectroscopy. Tissue samples (100–200 mg) were subjected to microwave digestion in nitric acid, and the resulting solutions were directly basified, brought to pH 7 in 20 mM HEPES buffer, and analyzed with MF1. Overall sample processing takes less than 15 min, and the assay is amenable

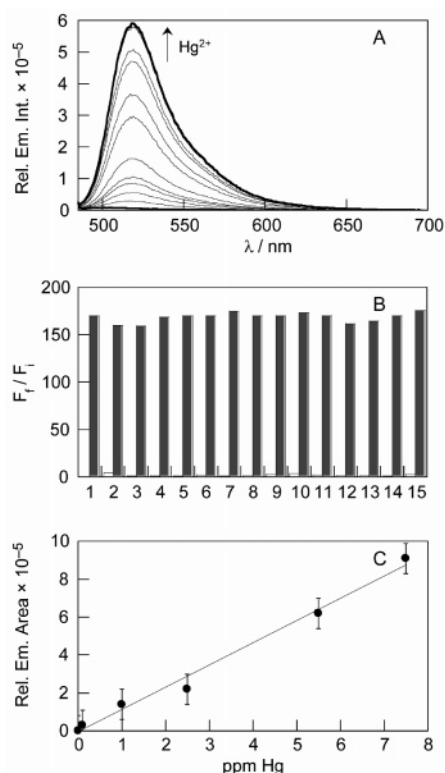


Figure 1. (A) Fluorescence response of 1 μM MF1 to Hg^{2+} in aqueous solution. Spectra shown are for Hg^{2+} concentrations of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.2, 1.6, and 2.0 μM . Spectra were acquired in 20 mM HEPES (pH 7) with excitation at 480 nm. (B) Fluorescence responses of MF1 to various metal ions. Bars represent the final integrated fluorescence response (F_f) over the initial integrated emission (F_i). Initial spectra were acquired in 20 mM HEPES, pH 7. White bars represent the addition of an excess of the appropriate metal ion (1 mM for Li^+ , Na^+ , K^+ , Mg^{2+} , and Ca^{2+} , 67 μM for all other cations) to a 1 μM solution of MF1. Gray bars represent the addition of 6.7 μM Hg^{2+} to the solution. Excitation was provided at 495 nm, and the emission was integrated over 505–700 nm. 1. Hg^{2+} ; 2. Li^+ ; 3. Na^+ ; 4. K^+ ; 5. Mg^{2+} ; 6. Ca^{2+} ; 7. Sr^{2+} ; 8. Mn^{2+} ; 9. Fe^{2+} ; 10. Co^{2+} ; 11. Ni^{2+} ; 12. Cu^{2+} ; 13. Zn^{2+} ; 14. Cd^{2+} ; 15. Pb^{2+} . (C) Fluorometric analysis of mercury in fish using MF1. Fish were taken from California waters and digested with microwave irradiation, and emission responses were calibrated versus independent measurement of mercury content by atomic absorption spectroscopy: Lime Saddle Marina (bluegill, 0.1 ppm Hg), Calero Reservoir (bass, 1.0 ppm Hg), Lake Almaden (bass, 2.5 ppm Hg), Almaden Reservoir (bass, 5.5 ppm Hg), Guadalupe Reservoir (bass, 7.5 ppm Hg). Excitation was provided at 495 nm, and the emission was integrated over 505–700 nm.

to parallel high-throughput screening methods. In addition, the small sample sizes employed are compatible with catch-and-release programs used for field studies²⁵ with no need for fish euthanization. The data collected in Figure 1C show a good linear correlation between emission response and total mercury content over a range of 0.1 to 8 ppm, establishing that MF1 is capable of distinguishing safe and toxic levels of mercury in edible fish samples according to the 0.55 ppm U.S. EPA standard.¹ This fluorescence method is complementary to colorimetric assays described previously.^{25,26}

In closing, we have described the synthesis, properties, and environmental applications of MF1, a unique fluorescent chemosensor for screening mercury in fish. MF1 exhibits excellent

selectivity for Hg^{2+} over competing environmentally relevant metal ions and the largest turn-on response to date for detecting this ion. Furthermore, MF1 is capable of measuring mercury levels in fish well within the safe edible limit. While demonstrated in fish, this method provides a useful starting point for developing new mercury contamination screens for a wide range of biological, toxicological, and environmental samples. The combined gains in selectivity and dynamic range presage many opportunities for MF1 and related Hg^{2+} chemosensors in laboratory and field applications.

Acknowledgment. We thank Richard Bauer (U.S. EPA), David Crane and Martice Vasquez (California Department of Fish and Game), and James Sanborn (California Office of Environmental Health Hazard Assessment) for providing fish samples. The University of California, the Camille and Henry Dreyfus Foundation, and the Arnold and Mabel Beckman Foundation generously supported this work. A.E.A. is a trainee of the Chemical Biology Graduate Program (T32 GM066698) and acknowledges a Berkeley Chancellor's Opportunity Fellowship. C.J.C. thanks Prof. Stephen Lippard and Ms. Elizabeth Nolan for many stimulating intellectual and practical discussions that inspired this research.

Supporting Information Available: Synthetic and experimental details (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA0557987