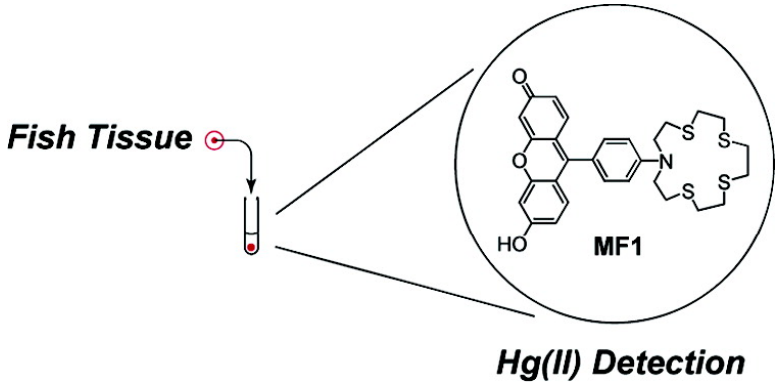
Communication

Screening Mercury Levels in Fish with a Selective Fluorescent Chemosensor

Sungho Yoon, Aaron E. Albers, Audrey P. Wong, and Christopher J. Chang

J. Am. Chem. Soc., **2005**, 127 (46), 16030-16031• DOI: 10.1021/ja0557987 • Publication Date (Web): 28 October 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 45 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 10/28/2005

Screening Mercury Levels in Fish with a Selective Fluorescent Chemosensor

Sungho Yoon, Aaron E. Albers, Audrey P. Wong, and Christopher J. Chang*

Department of Chemistry, University of California, Berkeley, California 94720

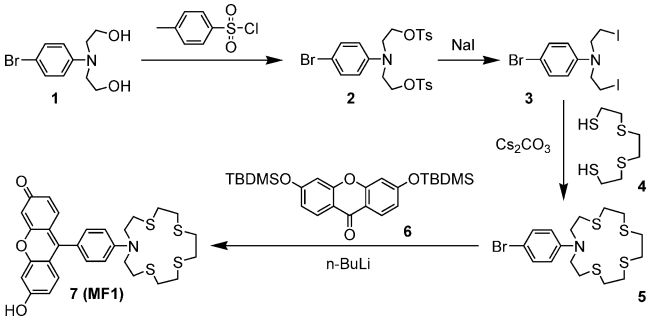
Received August 30, 2005; E-mail: chrischang@berkeley.edu

Mercury is a dangerous and widespread global pollutant.[1] The long atmospheric residence time of Hg^0 vapor and its oxidation to soluble inorganic Hg^{2+} 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 Hg^{2+} 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 Hg^{2+} from biological and environmental samples. Current techniques for mercury screening, including atomic absorption/emission spectroscopy[4] and inductively coupled plasma mass spectrometry,[5] often require expensive and sophisticated instrumentation and/or sample preparation. Fluorescence detection with Hg^{2+}-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 Hg^{2+} selectively over competing metal ion contaminants. Several types of small molecules,[6−18] DNAzymes,[19] and protein[20] or oligonucleotide[21] platforms have been examined for fluorescence Hg^{2+} detection. However, none of these probes have been utilized successfully for sensing Hg^{2+} in natural samples, as available Hg^{2+}-responsive fluorophores are often limited by nonspecific interference from Cu^{2+}, Pb^{2+}, and other competing metal ions, incompatibility with aqueous media, and/or delayed or irreversible Hg^{2+} 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 Hg^{2+} over competing analytes, including common metal ion contaminants Cu^{2+} and Pb^{2+}, and the largest fluorescence enhancement to date for sensing Hg^{2+} 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 reporter[22] having desirable optical properties and water solubility with a thioether-rich crown receptor to favor selective and stable binding of soft Hg^{2+} 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 Cs_2CO_3 under high-dilution conditions produces the azathiacrown receptor **5** in low yield (9%). Lithium-mediated coupling[24] 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 Hg^{2+}, MF1 has a visible absorption band centered at 485 nm ($\epsilon = 2.0 \times 10^4\ M^{-1}\ cm^{-1}$) 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 Hg^{2+}, 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, $\epsilon = 4.9 \times 10^4\ M^{-1}\ cm^{-1}$) and emission (517 nm) maxima. Binding analysis using the method of continuous variations establishes that a 1:1 Hg^{2+}: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 Hg^{2+} is obtained for MF1.

MF1 is highly selective for Hg^{2+} 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 Hg^{2+}-bound form are unperturbed by millimolar concentrations of Li^+, Na^+, K^+, Mg^{2+}, and Ca^{2+}, indicating excellent selectivity over these alkali and alkaline earth cations. MF1 is also selective for Hg^{2+} over first-row transition metal ions Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+} at 67-fold excess. The observed selectivity of MF1 for Hg^{2+} over Cu^{2+} is notable and reveals a greater affinity of the NS4 macrocycle for the former.[23] In addition, MF1 is also selective for Hg^{2+} over group 12 ions Zn^{2+} and Cd^{2+}, as well as the common heavy metal ion pollutant Pb^{2+}.

With a firm understanding of the spectroscopic properties and Hg^{2+} 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

10.1021/ja0557987 CCC: $30.25

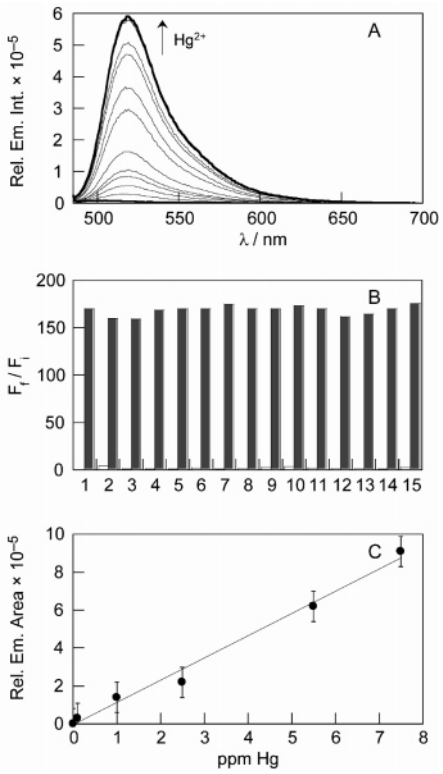


Figure 1. (A) Fluorescence response of 1 μM MF1 to Hg^{2+} in aqueous solution. Spectra shown are for Hg^{2+} concentrations of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.2, 1.6, and 2.0 μM. Spectra were acquired in 20 mM HEPES (pH 7) with excitation at 480 nm. (B) Fluorescence responses of MF1 to 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 Resevoir (bass, 1.0 ppm Hg), Lake Almaden (bass, 2.5 ppm Hg), Almaden Resevoir (bass, 5.5 ppm Hg), Guadelupe Resevoir (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 studies[25] 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.

References

(1) U.S. EPA, Regulatory Impact Analysis of the Clean Air Mercury Rule: *EPA-452/R-05-003*, 2005.
(2) Boening, D. W. *Chemosphere* **2000**, *40*, 1335−1351.
(3) Clarkson, T. W.; Magos, L.; Myers, G. J. *N. Engl. J. Med.* **2003**, *349*, 1731−1737.
(4) Bloom, N.; Fitzgerald, W. F. *Anal. Chim. Acta* **1988**, *208*, 151−161.
(5) Moreton, J. A.; Delves, H. T. *J. Anal. Atom. Spectrosc.* **1998**, *13*, 659−665.
(6) Chae, M. Y.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 9704−9705.
(7) Hennrich, G.; Sonnenschein, H.; Resch-Genger, U. *J. Am. Chem. Soc.* **1999**, *121*, 5073−5074.
(8) Rurack, K.; Kollmannsberger, M.; Resch-Genger, U.; Daub, J. *J. Am. Chem. Soc.* **2000**, *122*, 968−969.
(9) Prodi, L.; Bargossi, C.; Montalti, M.; Zaccheroni, N.; Su, N.; Bradshaw, J. S.; Izatt, R. M.; Savage, P. B. *J. Am. Chem. Soc.* **2000**, *122*, 6769−6770.
(10) Sakamoto, H.; Ishikawa, J.; Nakao, S.; Wada, H. *Chem. Commun.* **2000**, 2395−2396.
(11) Al Shihadeh, Y.; Benito, A.; Lloris, J. M.; Martinez-Manez, R.; Pardo, T.; Soto, J.; Marcos, M. D. *Dalton Trans.* **2000**, 1199−1205.
(12) Descalzo, A. B.; Martinez-Manez, R.; Radeglia, R.; Rurack, K.; Soto, J. *J. Am. Chem. Soc.* **2003**, *125*, 3418−3419.
(13) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 14270−14271.
(14) Guo, X.; Qian, X.; Jia, L. *J. Am. Chem. Soc.* **2004**, *126*, 2272−2273.
(15) Ros-Lis, J. V.; Marcos, M. D.; Martinez-Manez, R.; Rurack, K.; Soto, J. *Angew. Chem., Int. Ed.* **2005**, *44*, 4405−4407.
(16) Liu, B.; Tian, H. *Chem. Commun.* **2005**, 3156−3158.
(17) Mello, J. V.; Finney, N. S. *J. Am. Chem. Soc.* **2005**, *127*, 10124−10125.
(18) Nolan, E. M.; Lippard, S. J. *J. Mater. Chem.* **2005**, *15*, 2778−2783.
(19) Thomas, J. M.; Ting, R.; Perrin, D. M. *Org. Biomol. Chem.* **2004**, *2*, 307−312.
(20) Chen, P.; He, C. *J. Am. Chem. Soc.* **2004**, *126*, 728−729.
(21) Ono, A.; Togashi, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 4300−4302.
(22) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer Academic/Plenum: New York, 1999.
(23) Caltagirone, C.; Bencini, A.; Demartin, F.; Devillanova, F. A.; Garau, A.; Isaia, F.; Lippolis, V.; Mariani, P.; Papke, U.; Tei, L.; Verani, G. *Dalton Trans.* **2003**, 901−909.
(24) Minta, A.; Kao, J. P. Y.; Tsien, R. Y. *J. Biol. Chem.* **1989**, *264*, 8171−8178.
(25) Brummer, O.; La Clair, J. J.; Janda, K. D. *Bioorg. Med. Chem.* **2001**, *9*, 1067−1071.
(26) Brummer, O.; La Clair, J. J.; Janda, K. D. *Org. Lett.* **1999**, *1*, 415−418.

JA0557987