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An Organoselenium-Based Highly Sensitive and Selective Fluorescent "Turn-On" Probe for the Hg^{2+} Ion

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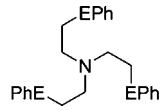
Supporting Information

ABSTRACT: An organoselenium-based NSe_3 type of tripodal system **2** as a Hg^{2+}-selective fluorescence "turn-on" probe is described. The "turn-on" fluorescence behavior of this selenotripod **2** is significant because it depends on Hg−Se bond formation and acts as a reporting unit for this system. The system exhibits immediate response (15 s) with a subnanomolar detection limit (0.1 nM) for the Hg^{2+} ion. It efficiently detects both aqueous and nonaqueous Hg^{2+} at 2 nM concentration.

In a continuation to our studies on organoselenium compounds, we also directed our attention toward their Hg^{2+} complexes, revealing a Hg−Se bond under certain considerations.[1] In a conventional paradigm on Hg^{2+} toxicity, mercury is antagonist for selenium. It should be noted that the selenoenzymes are required to prevent and reverse oxidative damage throughout human body cells and the mitigating role of selenium in mercury detoxification to a certain concentration is one of the well-accepted examples of biological antagonism.[2,3] The protective effect of selenium against mercury in human beings is believed to lead to the loss of selenoenzyme activities and their synthesis as a consequence of Hg^{2+}-based seizure of selenium. This may be further rationalized because of the fact that binding affinities of Hg^{2+} for selenium are up to a million times higher[4] than those of its second-best binding partner, i.e., sulfur.[5] Because of the selenophilic characteristics of Hg^{2+}, one may anticipate preferred selectivity and effective binding with selenium.[1,6] Despite the fundamental and biological significance of Hg−Se bond formation, the potential selenium compounds that can be used as an effective means of recognizing the Hg^{2+} ion or counteracting its toxicity have not been systematically studied. Indeed, the chemistry of Hg−Se-bonded species is limited because of either their insoluble nature or polymeric characteristics.[7] Therefore, identification of suitable organoselenium species and their specific interaction with Hg^{2+} is highly desirable. Moreover, remediation from the adverse effects related to Hg^{2+} species continues to remain a serious challenge.[8] Bearing all of these issues in mind, an interesting prospect that deserves deeper investigation is the sensitivity, selectivity, and entrapment behavior of organoselenium compounds toward the detection and detoxification of Hg^{2+} ions. The success of such an approach mainly depends on the identification of suitable organoselenium ligands and is yet to be realized. We herein report our preliminary investigations from an identified selenium-based tripod system **2** (Scheme 1) as highly selective and sensitive for binding Hg^{2+} ions even in the presence of several other chalcophilic metal ions. The tripod architecture[6,9] and flexibility of the donor arms of selenotripod **2** are highly favorable in achieving a monomeric mercury species in a predictable geometry. This study is further distinct from the fluorescence-based sensors because selenotripod **2** itself exhibits fluorescence and a "turn-on" behavior on Hg−Se bond formation.[10−12] This allows us to measure the Hg^{2+} ion concentration at nanomolar concentration using fluorescence spectroscopy.

Scheme 1. Systems Used in This Study



E = S **1**, Se **2**, Te **3**

The Hg^{2+}-sensing studies were carried out by using a $Hg(NO_3)_2$ salt at neutral pH under unbuffered conditions at 25 °C (298 K). The addition of an aqueous solution of $Hg(NO_3)_2$ to a solution of selenotripod **2** led to observable changes in the UV−visible spectra (Figure 1). The peak at λ = 266 nm in **2** exhibited an inhibition of absorbance, i.e., a hypochromic effect upon the addition of a Hg^{2+} solution. An isosbestic point at λ = 281 nm was also observed along with a hyperchromic effect at λ = 300 nm. The spectral changes were observed until attainment of a 1:1 equivalence between **2** and Hg^{2+}, as shown by Job's

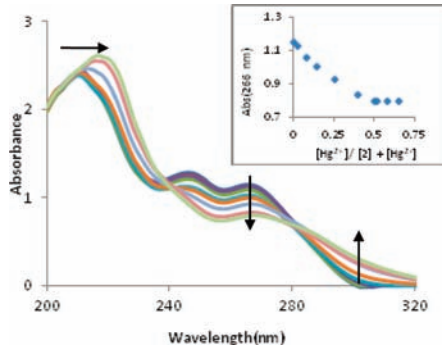


Figure 1. Absorption spectra of **2** (100 μM) in 80:20 (v/v) CH_3CN−H_2O and changes observed upon the addition of a $Hg(NO_3)_2$ salt solution (0−100 μM) in H_2O. Inset: Job's plot showing minima at 0.50, indicating 1:1 binding between **2** and Hg^{2+}.

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plot minima for $[Hg^{2+}]/([\mathbf{2}] + [Hg^{2+}])$ at 0.50, indicating the formation of a 1:1 species between system **2** and the Hg^{2+} ion in solution.

The fluorescence spectrum of **2** in acetonitrile−water (80:20) shows a weak emission at λ = 302 nm. However, a 4-fold enhancement in fluorescence was observed upon the addition of 1 equiv of an aqueous Hg^{2+} solution (Figure 2). The

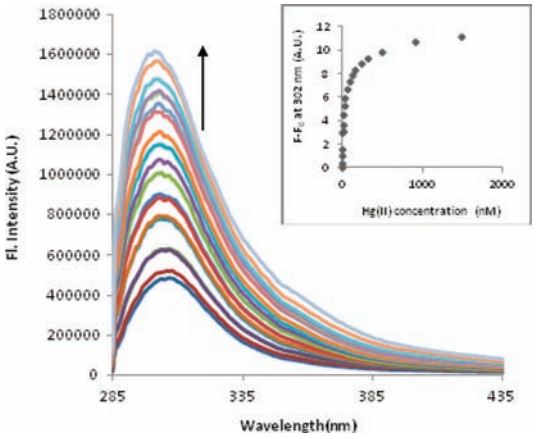

Figure 2. Emission spectra of **2** (1.5 μM) in 80:20 (v/v) CH_3CN-H_2O and changes observed upon the addition of a $Hg(NO_3)_2$ salt solution (0−1.5 μM) in H_2O. Inset: Enhancement in the fluorescence intensity observed at 302 nm upon the addition of a Hg^{2+} solution ($\lambda_{excitation}$ = 270 nm; $\lambda_{emission}$ = 285 nm).

fluorescence enhancement (10%) of system **2** for an aqueous solution of Hg^{2+} at the 2 nM level was a remarkable response over many of the fluorescence-based probes.[10,11] This further indicates that the Hg−Se bond formation process can be monitored by enhancement in fluorescence spectroscopy. Moreover, a linear response to Hg^{2+} in the concentration range 2−165 nM can be utilized at best for the practical estimation of the Hg^{2+} ion present in solution (Figure S9, SI). Further, in comparison to most of the other fluorescent probes reported to date for Hg^{2+} detection, an immediate fluorescence enhancement due to the Hg−Se bond was observed in the present case upon mixing of a Hg^{2+} solution with selenotripod **2** (15 s). Additionally, no appreciable changes in the emission spectra were noticed from 30 min to 24 h in all of the observed cases, which indicates that the initially formed species in solution is irreversible. The detection limit of this new chemodosimeter **2** was found to be 1.0×10^{-10} M (or 0.1 nM), which is 100 times less than the EPA standards (10 nM)[13] and is the lowest detection limit observed so far, lower than any chemodosimeter reported for Hg^{2+}-ion detection.[10] The anion effect on this Hg^{2+} sensing was also found negligible because fluorescence studies repeated with mercuric perchlorate and chloride salts under identical conditions revealed observations similar to those obtained with mercuric nitrate salt (Figure S10, SI). Over the pH range tested, a stable and sensitive fluorescence response in the pH range of 4.0−9.0 was observed, affording the possibility for detection of the Hg^{2+} ion by **2** under both acidic and basic conditions (Figures S11 and 12, SI). The Hg^{2+} species formed upon binding of selenotripod **2** with Hg^{2+} also exhibited thermal stability in the temperature range of 25−45 °C (Figure S13, SI). Further, no change in the fluorescence response was observed under oxidizing conditions (Figure S14, SI). For comparison purposes, sulfur (**1**) and tellurium (**3**) analogues of **2** have also been examined for their response toward Hg^{2+} ions. Comparatively, no significant changes were observed upon the addition of Hg^{2+} into a solution of **1** or **3** (Figures S15−17, SI).

Because the coordination chemistry of some of the chalcophilic metal ions (Ag^+, Cu^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, and Co^{2+}) with organoselenium donors has been studied earlier, the fluorescence response of system **2** toward possible interference of these metal ions along with K^+ and Fe^{3+} ions was also examined (Figure 3). No appreciable changes were

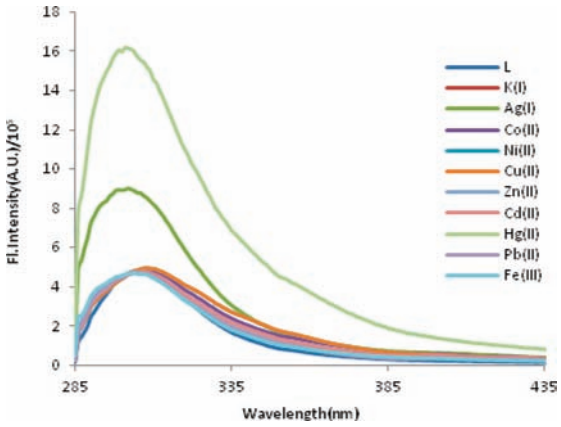

Figure 3. Changes observed in the emission spectrum of **2** (1.5 μM) in 80:20 (v/v) CH_3CN-H_2O upon the addition of various metal nitrate salts (25 equiv) in H_2O except Hg^{2+} (1 equiv) ($\lambda_{excitation}$ = 270 nm; $\lambda_{emission}$ = 285 nm).

observed upon the addition of even 25 equiv of these metal ions except Ag^+. A nearly 2-fold rise in fluorescence was observed upon the addition of 1 equiv of Ag^+, but no further changes were observed even upon the addition of up to 25 equiv of Ag^+. However, the addition of 1 equiv of a Hg^{2+} solution into this **2**·Ag^+-containing solution immediately showed fluorescence enhancement as observed for **2**·Hg^{2+}. These results are suggestive that Ag^+ bonded to **2** in solution can be exchanged by the Hg^{2+} ion. This was further confirmed separately when an isolated Ag^+ species with **2** in a 1:1 ratio dissolved in CH_3CN and treated with 1 equiv of Hg^{2+} led to isolation of **2**·Hg^{2+} species. The recovery of 99.9% Ag^+ ion was also confirmed by a solvent-extraction process. The other metal ions were also tested for their likely interference in Hg^{2+}-ion detection by **2**. In all of the cases, fluorescence enhancement of selenotripod **2** upon the addition of 1 equiv of the Hg^{2+} ion into a solution containing the other tested metal ions (25 equiv) was identical with that observed for the Hg^{2+} ion alone (Figure S18, SI).

The ^{77}Se NMR spectra of a 1:1 species of selenotripod **2** and Hg^{2+} species revealed a peak at δ 206.7 ppm (Figure S19, SI). This is a significant upfield shift in comparison to that of selenotripod **2** (δ 279.1 ppm)[14] and is in contrast to the usual downfield shift observed upon metal−ligand coordination. This may be attributed to a significant charge transfer between the mercury and selenium centers upon binding. This charge transfer between the mercury and selenium centers makes **2** a unique system to be directly monitored by fluorescence spectroscopy. No additional peaks were observed in the ^{77}Se NMR spectrum even at higher scans (10 K). The dissociation of the complex with time (7 days) as a consequence of the solvent-exchange effect was found negligible. It can be suggested that the existing species in solution is coordinatively

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intact. It is interesting to note here that, because of very high solvation of the mercury ion, their direct recognition in both aqueous and nonaqueous solutions is quite difficult. For example, in a solution of $HgCl_2$ (at 25 °C), an equilibrium between 12 mercury species was observed, namely, $HgCl_n^{2-n}$ with n = 0–4, HgOHCl, $Hg(OH)_n^{2-n}$ with n = 1–3, $HgOH^{3+}$, $Hg_3(OH)_3^{3+}$, and HgO(s).[15] Therefore, the solution obtained from the fluorescence titration experiment was further analyzed by mass spectroscopy to observe whether any mercury species formed in an aqueous Hg^{2+} solution had been left out of the estimation by selenotripod **2**. It exhibited a molecular-ion peak at m/z 933.7816 ($[\mathbf{2} + Hg(NO_3)_2 + K]^+$). However, no peak corresponding to any solvated mercury ion was observed in the mass spectrum. Moreover, the fluorescence experiment carried out with an acetonitrile solution of $Hg(NO_3)_2$ also provided a fluorescence enhancement response identical with that obtained with an aqueous $Hg(NO_3)_2$ solution (Figure S20, SI). In fact, the mass experiment repeated after 7 days also returned identical results. The structural determination of a crystal obtained from the solution upon slow evaporation was also attempted. The X-ray structure obtained from this crystal is shown in Figure 4. The three selenium-donor arms of **2**

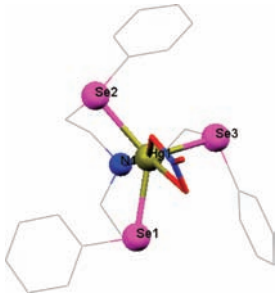

Figure 4. Molecular structure of the cationic portion of $[\mathbf{2}\cdot Hg(NO_3)]NO_3$ (hydrogen atoms and anionic NO_3 omitted for clarity).

coordinate to the Hg^{2+} metal center with similar Hg–Se bond distances (2.652–2.683 Å). These bond distances are significantly longer than the sum of the covalent radii of mercury and selenium (2.52 Å).[16]

The high selectivity or specificity for the Hg^{2+} ion (in absence of Ag^+ ion), low response time at ambient temperature (25 °C), and fluorescence "turn-on"-type behavior are remarkable. The present selenotripod **2** fulfills the required criteria for Hg^{2+}-ion detection and further supersedes over most of the fluorophore-containing Hg^{2+}-ion probes. The present system also holds the added advantages of being structurally and synthetically simple and economical. Undoubtedly, **2** may be taken as a unique system that exhibits direct fluorescence enhancement as a function of Hg–Se bond formation in solution, which is the first of its kind.

To summarize, we have identified and demonstrated a structurally and synthetically simple selenotripod **2** as a practical and economical Hg^{2+} fluorescent "turn-on" probe that does not require any external fluorophore as the reporting unit. The system has subnanomolar detection limit (0.1 nM) with an extremely low response time (15 s) at ambient temperature (25 °C).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic procedure and characterization data for **1**–**3**, scanned copies of spectral data of **2** and **3**, various emission spectra and other information mentioned in this Communication, and a CIF file of $\mathbf{2}\cdot Hg(NO_3)_2$. This material is available free of charge via the Internet at http://pubs.acs.org.

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