Synthesis and Characterization of a Rapid and Highly Selective Fluorescent Hg$^{2+}$ Sensor in Aqueous Media

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Received July 16, 2010, Accepted November 10, 2010

Key Words: Fluorescent chemosensor, Pyrene, Hg$^{2+}$ ion

Heavy metal ions pose risks to human health and environment. Among the metal ions, Hg$^{2+}$ is one of the most important toxic metal ions, because of its wide use in electrical equipment, catalysts, and paints, after which it is released into environment. Thus, it is highly desirable to develop a Hg$^{2+}$ sensor applicable for industrial waste sites and the environment. Among known metal ion sensors, fluorescent sensors have drawn much interest owing to their potential for on-site and real-time detection of toxic metal ions with low detection limits. A number of fluorescent sensors have been reported, including small molecules, whose quenching process is based on Hg$^{2+}$ coordination. To enhance the sensitivity, however, it is essential to design a sensor that does not quench fluorescence upon metal ion recognition. Although there have been reports of a fluorescent turn-on Hg$^{2+}$ sensor by several groups, still there are some drawbacks such as multiple synthesis steps from commercially available materials and low overall synthetic yield. Hence, it is desirable to develop new types of simple fluorescent sensors applicable in a variety of areas such as environmental monitoring and diagnostic analysis.

The pyrene is one of the most widely used fluorescent dyes in the design of fluorescent sensors for heavy metal ions. Because of strong thiophilic affinity of Hg$^{2+}$, carbamoyl thiourea derivatives have showed good binding property to Hg$^{2+}$ in fluorescent sensors. But there are still some drawbacks including interference from other metal ions or irreversibility. Hence, it is desirable to develop new types of simple fluorescent sensors applicable in a variety of areas such as environmental monitoring and diagnostic analysis.

In the present study, we report the synthesis and metal-binding properties of new pyrene-based Hg$^{2+}$ fluorescent sensor Py-1. The fluorescent Hg$^{2+}$ sensor Py-1 was prepared in one step from commercially available 1-pyrenemethylamine hydrochloride and benzoyl isothiocyanate in high yield. (Scheme 1) We found that this turn-on sensor Py-1 exhibits a rapid response and an extremely good selectivity toward Hg$^{2+}$ compared with other metals in aqueous solution. We believe that it could have application in a variety of areas, such as environmental monitoring and diagnostic analysis.

Py-1 was prepared according to Scheme 1. Under argon, a solution of 1-pyrenemethylamine hydrochloride (0.268 g, 1 mmol), benzoyl isothiocyanate (0.163 g, 1 mmol) in CH$_3$CN (20 mL) was refluxed for 36 h. Then 30 ml CHCl$_3$ was added, and the solution was filtered. The filtrate was removed by rotary evaporation to produce a yellow-orange solid. The molecular structure of Py-1 was confirmed by mass spectrometry and NMR. Fluorescence spectra were recorded with a FL-4500 fluorometer. Stock solutions (1.00 mM) of metal salts were prepared in H$_2$O/CH$_3$CN (1:1, v/v). Stock solutions of free Py-1 (1.00 mM) were prepared in CH$_3$CN.

The effects of metal ion addition on the absorption and fluorescence properties of Py-1 in CH$_3$CN/H$_2$O (1/1) were investigated to evaluate the metal ion binding properties of the sensor. We did not observe any changes in absorption spectra upon addition of metal ions except for Ag$^+$. The fluorescence spectra of Py-1 (c = 10$^{-5}$ M) were recorded following 342 nm excitation. Figure 1 shows the fluorescence spectra of Py-1 upon the addition of various metal ions, Ag$^+$, Cd$^{2+}$, Cu$^{2+}$, Fe$^{3+}$, Ni$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, and Hg$^{2+}$. In the absence of metal ion, Py-1 exhibited a very weak fluorescence intensity compared with that of pyrene owing to efficient photo-induced electron transfer from the metal binding site to the fluorescent unit. When Hg$^{2+}$ was introduced to a 1 μM Py-1, a 23-fold fluorescence intensity enhancement was observed, a result of inhibition of the PET quenching pathway. On the other hand, little fluorescence enhancement

![Figure 1. Fluorescence spectra of fluorescent sensor Py-1 (1 μM) in H$_2$O/CH$_3$CN (1:1, v/v) in the presence of Hg$^{2+}$, Ag$^+$, Cd$^{2+}$, Cu$^{2+}$, Fe$^{3+}$, Ni$^{2+}$, Pb$^{2+}$, Zn$^{2+}$ (10 equiv).](image-url)
was observed for other metal ions. These results indicate that the fluorescence sensor Py-1 exhibits a good selectivity toward Hg$^{2+}$ over other metals in aqueous solutions. The fluorescence spectrum of Py-1 in the presence of Hg$^{2+}$ exhibits similar to that of pyrene monomer, indicating that there is no dimer formation upon Hg$^{2+}$ ion complex.

Titration experiments were performed with 1.0 μmol solutions of fluorescent sensor Py-1 and various concentrations of Hg$^{2+}$ in H$_2$O/CH$_3$CN (1:1, v/v). Figure 2 illustrate the fluorescence titration spectra of Py-1 (1 μM) upon addition of various amounts of Hg$^{2+}$ (0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 equiv). It can be seen that the fluorescence intensity increases continuously with increasing Hg$^{2+}$ concentration. The inset shows the fluorescence titration profile of Py-1 at 390 nm upon addition of Hg$^{2+}$. The maximum point appears at the mole fraction of 0.60 in a Job’s plot, suggesting that the Py-1 forms a 2:1 ligand-to-metal complex. Mass spectrometry data also confirmed the formation of 2:1 complex. To elucidate the binding mechanism of Py-1-Hg$^{2+}$ complex, IR spectrum was measured in solid form. Upon addition of 10 equivalents of Hg$^{2+}$, the amide carbonyl band (1698 cm$^{-1}$) of Py-1 shifts to a lower frequency (1633 cm$^{-1}$), indicating that the amide carbonyl participates in coordination with Hg$^{2+}$. In addition, $^1$H NMR spectra also showed that the proton on thioamide was disappeared upon addition of Hg$^{2+}$, which indicate that the strong interactions between Hg$^{2+}$ and the sulfur atom of the thioamide group. To examine the reversibility of Py-1, excess of EDTA was added to the Py-1-Hg$^{2+}$ complex that exhibited weak fluorescence intensity, demonstrating reversibility to free Hg$^{2+}$ ion.

Figure 4 represents the selectivity and competition property of fluorescent sensor Py-1 over the metal ions of interest (Gray bars: addition of 10 equivalents of Ag$^+$, Cd$^{2+}$, Cu$^{2+}$, Fe$^{3+}$, Ni$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, Hg$^{2+}$ to the solution; Black bars: addition of 10 equiv of Ag$^+$ + Hg$^{2+}$, Cd$^{2+}$ + Hg$^{2+}$, Cu$^{2+}$ + Hg$^{2+}$, Fe$^{3+}$ + Hg$^{2+}$, Ni$^{2+}$ + Hg$^{2+}$, Pb$^{2+}$ + Hg$^{2+}$, Zn$^{2+}$ + Hg$^{2+}$, Cd$^{2+}$ + Hg$^{2+}$, respectively, in H$_2$O/CH$_3$CN (1:1, v/v)). All data (F) were normalized with respect to the emission of the free dye (F$_o$). According to competition experiments, other metal ions did not show any interference with Hg$^{2+}$ selectivity. Even addition of paramagnetic Ni$^{2+}$, Cu$^{2+}$, and Fe$^{3+}$, which are well-known fluorescence quencher, did little to quench fluorescence. These results demonstrate the applicability of the fluorescent sensor Py-1 for Hg$^{2+}$ specific sensing in the environment.

In conclusion, we have synthesized a pyrene-based fluorescent sensor, Py-1, and characterized its metal-binding properties. We found that fluorescent sensor Py-1 exhibits a rapid response and an extremely good selectivity toward Hg$^{2+}$ over other metal ions in aqueous solution.

Acknowledgments. This work was supported by Mid-career Researcher Program through NRF grant funded by the MEST (No. 2009-0083867).

References


Notes

Figure 2. Fluorescence titration spectra of fluorescent sensor Py-1 (1 μM) in H$_2$O/CH$_3$CN (1:1, v/v) upon addition of various amounts of Hg$^{2+}$ (0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 equiv).

Figure 3. Proposed geometry of the complexes.

Figure 4. Selectivity and competition property of fluorescent sensor Py-1 over the metal ions of interest. Gray bars: addition of 10 equiv of Ag$^+$, Cd$^{2+}$, Cu$^{2+}$, Fe$^{3+}$, Ni$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, Hg$^{2+}$ to the solution; Black bars: addition of 10 equiv of Ag$^+$ + Hg$^{2+}$, Cd$^{2+}$ + Hg$^{2+}$, Cu$^{2+}$ + Hg$^{2+}$, Fe$^{3+}$ + Hg$^{2+}$, Ni$^{2+}$ + Hg$^{2+}$, Pb$^{2+}$ + Hg$^{2+}$, Zn$^{2+}$ + Hg$^{2+}$, respectively, in H$_2$O/CH$_3$CN (1:1, v/v)).

Notes

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15. $^1$H NMR (CDCl$_3$, 300 MHz) 5.60 (d, 2H, $J = 3$ Hz), 7.46 (t, 2H, $J = 7.8$ Hz), 7.58 (t, 1H, $J = 7.5$ Hz), 7.77 (d, 2H, $J = 7.8$ Hz), 8.00-8.11 (m, 4H), 8.16-8.22 (m, 4H), 8.30 (d, 1H, $J = 9.3$ Hz), 9.12 (brs, 1H), 11.12 (brs, 1H). $^1$C NMR (CDCl$_3$, 75 MHz) 48.3, 122.6, 124.6, 124.8, 125.0, 125.4, 125.5, 126.1, 127.2, 127.3, 127.7, 128.5, 129.0, 129.1, 129.2, 130.7, 131.2, 131.5, 133.5, 166.7, 179.5. HRMS (70 eV, EI): $m/z$ calcd for C$_{25}$H$_{18}$N$_2$OS [M+] 394.1140, found 394.1135.