ORGANIC LETTERS

2011
Vol. 13, No. 19
5028–5031

Selective and Sensitive Ratiometric Detection of Hg(II) Ions Using a Simple Amino Acid Based Sensor

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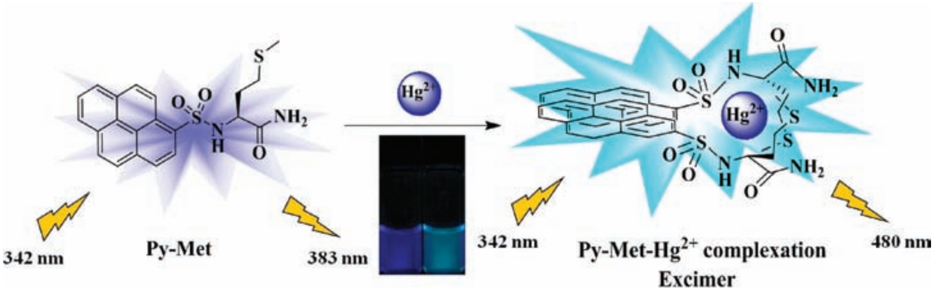
Received July 5, 2011

ABSTRACT



Synthesis of a novel pyrene derivative sensor (Py-Met) based on amino acid and its fluorescent behavior for Hg(II) in water was investigated. Upon Hg(II) binding, the Py-Met-bearing sulfonamide group exhibited a considerable excimer emission at 480 nm along with a decrease of monomer emission at 383 nm. Py-Met allows a selective and sensitive ratiometric detection of Hg(II) without any interference from other metal ions.

The design and synthesis of artificial receptors for sensing and recognition of heavy- and transition-metal ions have received considerable attention because these types of metal ions are toxic for humans and other living organisms.[1,2]Mercury ions are especially toxic: they contaminate the environment and cause serious problems for human health and ecology.[3] Fluorescent chemical sensors have consequently been developed as a means of detecting Hg(II) ions in environmental and biological samples.[4−8] There have been some successful achievements in the development of fluorescent chemical sensors for Hg(II). However, most of the reported Hg(II) sensors have shortcomings, particularly in terms of sensitivity, selectivity, interference from other metal ions, the turn-off response, and low solubility in aqueous solution.[4a]

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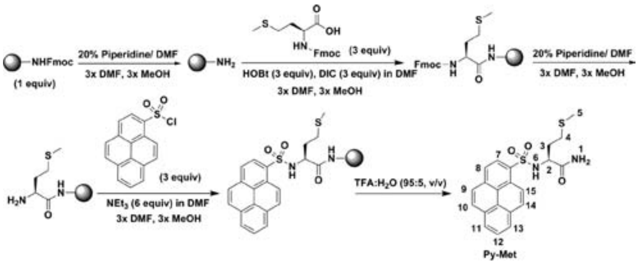
10.1021/ol201683t

Ratiometric sensing is highly recommended because ratiometric calibration accurately quantifies the analytes.[9] However, to date, the majority of mercury sensors detect Hg(II) by using either a turn-off response or a turn-on response. Surprisingly, only a few of the reported fluorescent sensors rely on a ratiometric response to Hg(II).[10] However, almost all ratiometric Hg(II) sensors suffer from Ag(I) or Cu(II) interference and low sensitivity.[10b–h] Some of them required a high proportion of organic solvent in media for ratiometric response.[10f–i] The development of a new ratiometric fluorescent sensor that can selectively and sensitively detect Hg(II) is therefore a highly challenging task.

Amino acids are highly water-soluble and environmentally compatible. Accordingly, the synthesis of amino acid-based chemical sensors has potential for the detection of metal ions in aqueous solution. Most chemical sensors based on amino acids responded sensitively to specific heavy metal ions in aqueous solutions and mixed aqueous/organic solutions.[11,12] We also recently demonstrated that Met-based chemical sensor bearing dansyl fluorophore responded in a highly selective manner to Hg(II) in aqueous solution.[11e] In this work, we extended our studies to the synthesis of a new simple ratiometric fluorescent sensor for Hg(II). Pyrene fluorophore was used in the design of the ratiometric sensor because the self-assembled complex of pyrene-conjugated sensors causes a distinctive change in a fluorescence emission spectrum on account of the pyrene excimer emission.[13] A metal ion-induced deprotonation of sulfonamide is an interesting phenomenon used in the design of selective metal ion chemosensors.[14] Sulfonamide group in several chemical sensors played a critical role in the recognition of metal ions.[5f,11c–11f,14b,14c] Thus, pyrenesulfonic acid was attached to the N-terminal of L-Met, as shown in Scheme 1. **Py-Met** was easily synthesized in a solid-phase synthesis with a high yield (78%). After cleavage of the product from resin, the compound was purified using semipreparative HPLC with a C18 column. The synthesis and high level of purity (>98%) of **Py-Met** were confirmed by using analytical HPLC and ESI mass spectrometry (Figure S1, Supporting Information). The structure of **Py-Met** was confirmed by using IR and ^{1}H and ^{13}C NMR spectroscopy.

Scheme 1. Synthesis of **Py-Met**



Py-Met as a chemosensor has four significant advantages besides its easy synthesis using solid-phase synthesis: first, it has a ratiometric response to mercury ions in aqueous solution; second, it has exclusive selectivity for Hg(II) and detects Hg(II) without interference from other metal ions such as Ag(I) and Cu(II); third, it responds rapidly and with a high level of sensitivity to nanomolar concentration of Hg(II); fourth, the sensor is suitable for monitoring Hg(II) in a broad pH range (4.5–11.5).

The fluorescent behavior of **Py-Met** was dependent on solvent (Figure S2, Supporting Information). As shown in Figure 1, the emission spectra of **Py-Met** were measured in 10 mM HEPES buffer solution containing DMF (98:2, v/v) at pH 7.4. **Py-Met** shows exclusive selectivity for Hg(II) among the test metal ions (Na(I), K(I), Mg(II), and Al(III) as chloride anion and Ag(I), Cd(II), Co(II), Hg(II), Cr(III), Ni(II), Fe(II), Cu(II), Pb(II), and Zn(II) as perchlorate anion) The spectrum of only **Py-Met** shows typical emission bands at 383 and 402 nm. These bands are attributed to the pyrene monomeric emission. The addition of Hg(II) to a solution of **Py-Met** leads to a significant decrease in the emission bands of the pyrene monomer and a considerable increase in the emission band of pyrene excimer fluorescence at 480 nm. The typical pyrene excimer emission band was observed with a clear isoemissive point at 443 nm.[13] This result indicates that two **Py-Met** interact with Hg(II) and then the pyrene fluorophore moieties are stacked. As a result, the emission intensities of the pyrene excimer are increased, and the intensity of the monomer is decreased. In the titration curve, the intensity ratio (I_{480}/I_{383}) at 480 and 383 nm increases as the Hg(II) concentration increases (Figure S3, Supporting Information). The intensity ratio (I_{480}/I_{383})

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changed significantly from 0.002 to 0.391 (ca. 195 fold) by adding Hg(II). A complete change in the emission intensity requires about 0.7 equiv of Hg(II). This value suggests that **Py-Met** has a sensitive response to Hg(II) and that **Py-Met** may form a 2:1 complex with Hg(II). During the UV/vis titration of mercury ions to **Py-Met**, a significant decrease of the absorbance at 352 nm and hypochromic shift of the absorption spectra were observed (Figure S4, Supporting Information). This is in agreement with the formation of pyrene dimers in the ground state.[15] These results strongly demonstrated that a pyrene dimer is formed upon binding of Hg(II) to **Py-Met**. ESI mass spectrum provides additional evidence of a 2:1 complex of **Py-Met** with Hg(II) (Figure 2). When 1.0 equiv of Hg(II) was added to the **Py-Met** solution, a new peak appeared at 1023.08 (*m*/*z*), which corresponds to [2**Py-Met** + **Hg(II)** − **H**]$^+$. On the basis of these results, it is concluded that the fluorescence response of **Py-Met** results from the formation of 2:1 complex with Hg(II).

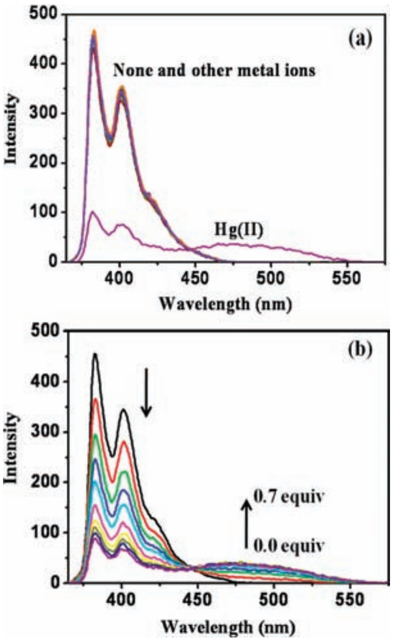

Figure 1. (a) Fluorescence response of **Py-Met** (20 μM) in the presence of various metal ions (1 equiv) and (b) fluorescence changes of **Py-Met** (20 μM) in the presence of various concentrations of Hg(II) (0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 equiv) in H_2O/DMF (98:2, v/v, 10 mM HEPES) at pH 7.4. (λ_{ex} = 342 nm).

Assuming a 2:1 complex formation, the association constant was calculated on the basis of the titration curve (Figure S5, Supporting Information). The association constant, K_a, of **Py-Met** for Hg(II) was calculated as 7.84×10^{12} M^{-2} (R^2 = 0.926); this value indicates that **Py-Met** has a potent binding affinity for Hg(II) in H_2O/DMF (98:2, v/v, 10 mM HEPES).

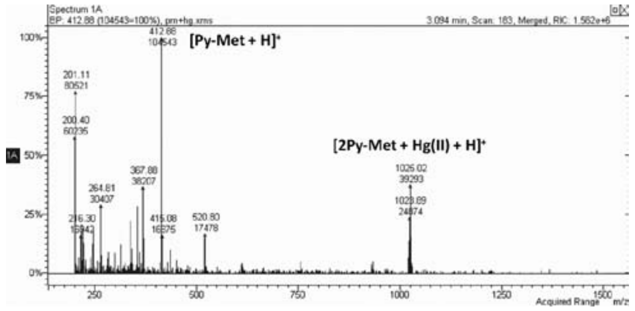

Figure 2. ESI mass spectrum of **Py-Met** (600 μM) in H_2O/CH_3CN (50:50, v/v) including $Hg(ClO_4)_2$ (1 equiv).

We plotted the emission intensity ratio (I_{383}/I_{480}) as the concentration of Hg(II) (Figure S6, Supporting Information). The emission intensity ratio decreased linearly as Hg(II) concentration increases from 0 nM to 750 nM. **Py-Met** has a detection limit of 57.2 nM (R^2 = 0.997) based on 3σ/m, where σ is the standard deviation of the blank measurements, and m is the slope of the intensity ratio versus sample concentration plot. This represents the lowest reported detection limit of Hg(II) by a ratiometric sensor.

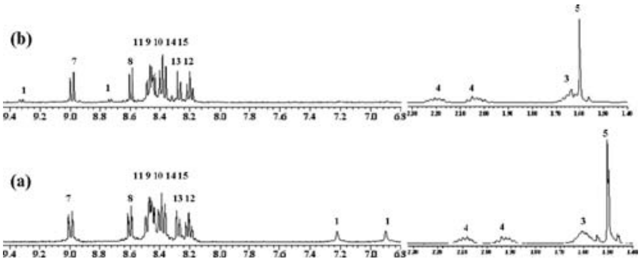

Figure 3. Partial ^{1}H NMR spectra of **Py-Met** (7.5 mM) in DMSO-d_6/D_2O (95:5, v/v): (a) free **Py-Met**; (b) **Py-Met** in the presence of $Hg(ClO_4)_2$ (0.5 equiv).

^{1}H NMR studies provide direct evidence of the interaction between **Py-Met** and Hg(II) (Figure 3). ^{1}H NMR experiments were carried out in DMSO-d_6/D_2O (95:5, v/v) because 7.5 mM of **Py-Met** dissolved well in this solvent system and **Py-Met** showed a fluorescent response to Hg(II) in this solvent system. When 0.5 equiv of $Hg(ClO_4)_2$ was added, the two protons in the amide group displayed large downfield shifts. These shifts can be attributed to the shielding effect, arising from the N−Hg(II) complexation. At the same time, the aliphatic protons, around the

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thioether group, displayed considerable downfield shifts. This is due to the interaction of the thioether group ($-SCH_3$) with Hg(II). The sulfonamide proton peak was not observed in this solvent condition. Thus, to confirm the role of the sulfonamide in **Py-Met**, we synthesized the analogue of **Py-Met** that had an amide group instead of the sulfonamide group. As expected, the analogue of **Py-Met** did not show any response to metal ions including Hg(II) (Figure S7, Supporting Information), which confirms that the sulfonamide group of **Py-Met** plays a critical role in the interaction with Hg(II). The sensitive and selective response of **Py-Met** for Hg(II) may be achieved by interactions of Hg(II) with the thioether group, the sulfonamide group, and the amide group.

The effect of pH influence on the fluorescence response of **Py-Met** to Hg(II) was examined (Figure S8, Supporting Information). The intensity at 383 nm of free **Py-Met** decreased as pH increased above 8.5, which may be due to the deprotonation of the sulfonamide group ($pK_a \approx 10$).[16] However, considerable increase of excimer emission intensity at 480 nm in the presence of Hg(II) was observed in the wide pH range (4.5−11.5). This result reveals that the 2:1 complex formation of **Py-Met** with Hg(II) occurs in this pH range. **Py-Met** is suitable for monitoring Hg(II) in a broad pH range (4.5−11.5).

Figure 4. Color emission changes of **Py-Met** (20 μM) upon addition of 1 equiv of metal ions in H_2O/DMF (98:2, v/v, 10 mM HEPES) at pH 7.4.

As shown in Figure 4, **Py-Met** shows a green color in the presence of 1 equiv of Hg(II) under UV light but a blue color in the presence or absence of other metal ions.

To investigate the interference effect of other metal ions on the detection ability of **Py-Met**, we measured the response of **Py-Met** to Hg(II) in the presence of other metal ions (Figure 5). The presence of other background metal ions did not show any obvious disturbance with the signal response induced by **Py-Met**−Hg(II) complexation. Specially, the ratiometric fluorescence response (I_{480}/I_{383}) of **Py-Met** for Hg(II) is unaffected by 1 mM of Group I and Group II metal ions. Most of the reported ratiometric sensors for Hg(II) display cross-sensitivities toward other heavy metal ions such as Ag(I) and Cu(II).[10b−h] However, **Py-Met** shows a reliable ratiometric detection for Hg(II) even in the presence of competing metal ions such as Ag(I) and Cu(II).

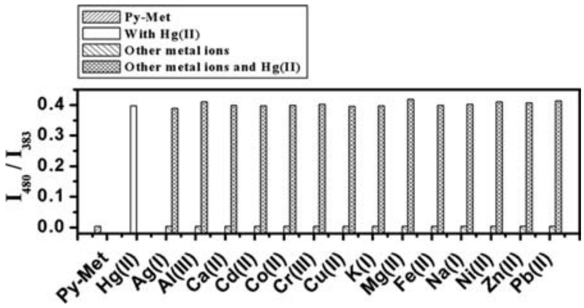

Figure 5. Fluorescence response of **Py-Met** (20 μM) in the presence of Hg(II) ions (1 equiv) and additional metal ions (1 equiv) in H_2O/DMF (98:2, v/v, 10 mM HEPES) at pH 7.4.

In conclusion, we present a new simple fluorescent ratiometric sensor for Hg(II). **Py-Met** can be easily synthesized in solid phase with a high yield. **Py-Met** with high binding affinity for Hg(II) (K_a, 7.84×10^{12} M^{-2}) shows highly selective and sensitive response to mercury ions in water. In addition, **Py-Met** is suitable for monitoring Hg(II) in a broad pH range (4.5−11.5) and detects Hg(II) without any interference from other metal ions. We expect that **Py-Met** may provide a potential tool for the detection and quantification of Hg(II) ions in environmental samples through its ratiometric response.

Acknowledgment. This work was supported by a grant (2009-0076572) from the Basic Research Program of National Research Foundation of Korea.

Supporting Information Available. Fluorescence emission spectra upon titration with Hg(II), ESI mass spectra with Hg(II), fluorescence emission intensity with Hg(II) in different pH, fluorescence emission spectra of the analogue of Py-Met, UV/vis spectra, and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.