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Turn-on Fluorescent Chemosensor Based on an Amino Acid for Pb(II) and Hg(II) Ions in Aqueous Solutions and Role of Tryptophan for Sensing

Lok Nath Neupane, Joo-Young Park, Ju Hun Park, and Keun-Hyeung Lee*

Bioorganic Chemistry Laboratory, Department of Chemistry, Inha University, Yunghyun-dong, Nam-gu, Incheon, 402-751, Republic of Korea

leekh@inha.ac.kr

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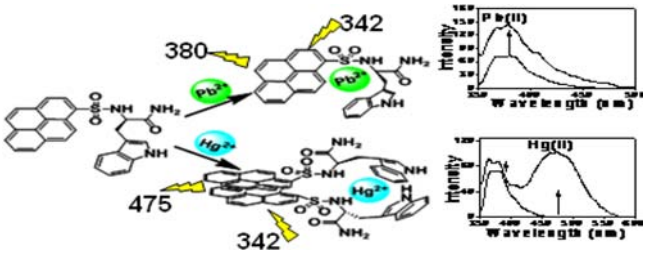
ABSTRACT



This communication presents a fluorescent chemosensor for detecting Pb(II) and Hg(II) in aqueous solutions. The sensor showed a turn-on response to Pb(II) by an enhancement of emission intensity at 380 nm and to Hg(II) by an enhancement of emission intensities at 380 and 475 nm. We have first characterized a unique function of tryptophan as a ligand as well as a quencher for recognition and fluorescent change by a metal binding event.

Selective and sensitive detection of toxic heavy and transition metal ions has been of great interest because these metal ions have caused adverse health and environmental problems. Especially, Hg(II) and Pb(II) have been regarded as the most toxic metal ions.[1,2] Even though some powerful analytical methods such as inductively coupled plasma mass spectrometry and atomic absorption spectrometry are currently used to monitor low levels of these metal ions, they have some drawbacks such as being time-consuming and expensive. Thus, a cheap and simple method for monitoring these metal ions is in high demand.[3,4] As fluorescence is the most efficient approach to detect low concentrations of analytes, there are many efforts devoted to the development of small fluorescent chemical sensors for Hg(II) or Pb(II).[5,6] Even though many different kinds of small chemical sensors for Hg(II) or Pb(II) have been reported, most of them suffer from limitations such as poor water solubility, low sensitivity, and low selectivity.[7−9] In general, the turn-on response for detecting metal ions is highly preferable in practical applications because the turn-off response can experience interference by other external factors.[10] Thus, the synthesis of new fluorescent chemosensors that selectively and sensitively detect heavy metal ions in aqueous solutions by a turn-on response is highly challenging.

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Fluorescent chemosensors consist of a fluorophore moiety and a receptor moiety. The fluorophore of chemosensors provides the fluorescence change by the binding event of analytes. The fluorescence change is induced by either photoinduced electron transfer (PET), fluorescence resonance energy transfer (FRET), monomer/excimer formation, or photoinduced charge transfer, etc.[5,6,11] As fluorescent chemosensors have been synthesized based on a hydrophobic fluorophore, most fluorescent chemosensors required a high proportion of organic solvent in media for proper operation due to low solubility in aqueous solutions. In recent years, hydrophilic biomolecules such as amino acids, peptides, and DNA have been used as a receptor for fluorescent chemosensors because these molecules had potent binding affinities to specific metal ions, biological compatibility, and high water solubility.[12−15] The chemosensors based on these biomolecules showed sensitive responses to heavy metal ions in aqueous solutions.

Among natural amino acids, tryptophan (Trp) is well-known to its intrinsic fluorescent property. As Trp fluorescence is sensitively influenced by local polarity, the fluorescence has been used to investigate protein conformation and folding.[16] In addition, various Trp containing peptides have been synthesized for investigating conformations by using FRET between Trp (donor) and the fluorophores (acceptor).[17] However, it is not well-known that Trp acts as quencher for specific fluorophores by long-range electron transfer due to a readily oxidized indole group.[18] Even the quenching effect of Trp on Alexa fluorophores, frequently attached to proteins, was recently characterized.[18c] Even though several fluorescent chemosensors containing Trp have been synthesized,[15,17a] the quenching effect of Trp on the fluorophores has not been observed and characterized.

In the present study, we present a new chemosensor based on Trp for monitoring Pb(II) and Hg(II) in aqueous solutions and have first reported a unique function of Trp as a ligand for the metal ions as well as a quencher for the fluorescent change by the metal binding event. In more detail, the binding of Pb(II) or Hg(II) to the Trp moiety of the chemosensor inhibits photoinduced electron transfer from the indole moiety to the pyrene fluorophore, resulting in a turn-on response.

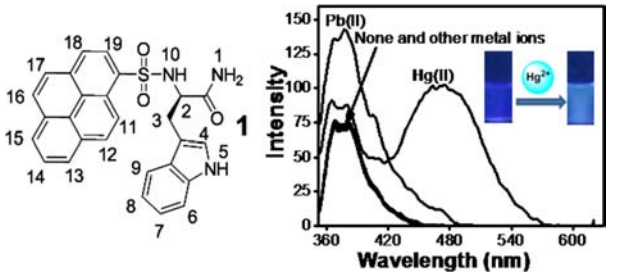

Figure 1. Structure and fluorescence emission spectra of **1** (10 μM) in the presence of metal ions (1 equiv) in HEPES buffer solution at pH 7.4.

Compound **1** was easily synthesized in 79% yield using a solid-phase synthesis (Figures S1−S6; see Supporting Information). The L-Trp amino acid was chosen as a receptor moiety and pyrene was selected as a fluorophore because pyrene dimerization causes a distinctive increase in excimer emission at 480 nm and a decrease of the pyrene monomer at 385 nm.[11,19]

The emission spectra of **1** were measured in aqueous solution (10 mM HEPES, pH = 7.4) containing 5% CH_3CN (Figure 1). Compound **1** itself has typical pyrene monomer bands at 378 and 395 nm in the absence of metal ions. **1** shows turn-on responses to Pb(II) and Hg(II) among 14 metal ions (Na(I), K(I), Mg(II), Al(III), Ag(I), Cd(II), Co(II), Hg(II), Cr(III), Ni(II), Fe(II), Cu(II), Pb-(II), and Zn(II)). Interestingly, **1** differentiates Pb(II) and Hg(II) by emission spectra changes. Figure 2 shows the fluorescence spectrum changes of **1** to the amount of Pb(II)

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and Hg(II), respectively. Upon the addition of Pb(II), the intensity of the monomer emission bands increased. Approximately 25 equiv of Pb(II) were required for the saturation of the emission intensity change, and the maximum emission intensity showed a ca. 7-fold enhancement. Upon the addition of Hg(II), a considerable increases in excimer emission and pyrene monomer emission were observed as the concentration of Hg(II) increased. The excimer emission intensity at 475 nm significantly increased from 1.00 to 80.0 (ca. 80). A complete change in the emission intensity requires only about 1.0 equiv of Hg(II), which suggests that **1** may have more potent binding affinity to Hg(II) than Pb(II) in aqueous solutions.

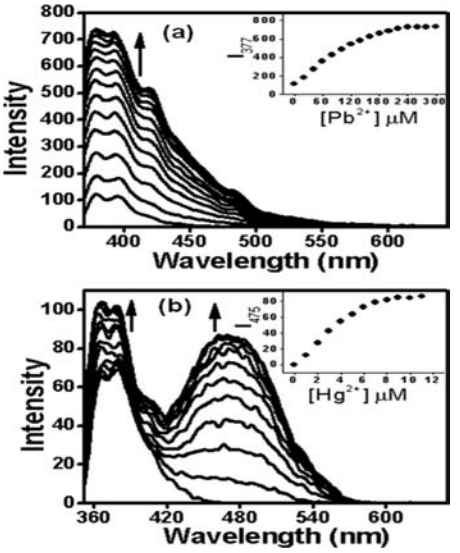

Figure 2. Fluorescence emission spectra of **1** (10 μM) upon addition of (a) Pb(II) and (b) Hg(II) in 10 mM HEPES buffer solution at pH 7.4 containing 5% CH_3CN (λ_{ex} = 342 nm, slit = 15/12 nm).

The complexation of **1** with the metal ion was investigated by absorption spectroscopy (Figure S7). The absorbance at 352 nm increased by adding Pb(II), whereas the addition of Hg(II) induced a significant decrease of the absorbance at 352 nm, which is attributable to stacked dimerization of the two pyrene fluorophore.[19] The visible emission changes of **1** in the presence of various metal ions were monitored by naked eyes under UV irradiation (Figure S8). The sample solution containing Hg(II) shows a different and brighter color than those of the sample solution containing the other metal ions.

We investigated the interference effect of the other metal ions on the ability of **1** to detect Hg(II) because **1** may show a potent binding affinity to Hg(II) among the tested metal ions. As shown in Figure 3, the Hg(II)-dependent fluorescence emission intensity of **1** was not considerably changed by the presence of other metal ions. Most of the reported turn-on mercury sensors that operated in aqueous solutions displayed cross-sensitivities toward heavy metal ions such as Cu(II) and Ag(I).[7a−e] The result demonstrates that **1** can detect Hg(II) in aqueous solution by a turn-on response without interference from the other metal ions except Pb(II).

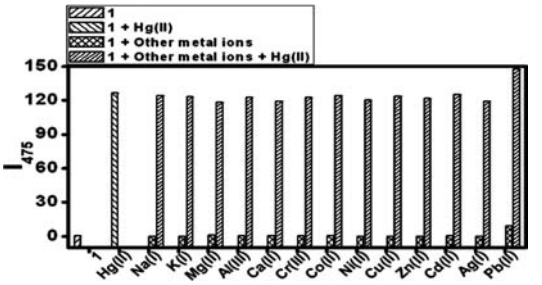

Figure 3. Emission intensity of **1** (10 μM) in the presence of Hg(II) ions (1 equiv) and additional metal ions (1 equiv) in 10 mM HEPES buffer solution at pH 7.4 containing 5% CH_3CN (λ_{ex} = 342 nm, slit = 15/13 nm).

In general, pyrene excimer emission at 480 nm increases, resulting in the decrease of pyrene monomer emission at 390 nm.[11,19] Interestingly, fluorescent titrations of **1** with Hg(II) shows that excimer emission at 480 nm increased with the enhancement of pyrene monomer emission at 390 nm. To obtain information about the binding mode, the binding stoichiometry was investigated (Figure S9). The maximum point at 0.33 and 0.5 for Hg(II) and Pb(II) in Job's plots indicates that **1** forms a 2:1 complex with Hg(II) and a 1:1 complex with Pb(II), respectively. ESI mass spectrometry was used to investigate the binding stoichiometry (Figure. 4). When Pb(II) or Hg(II) was added to the solution of **1**, respectively, a new peak appeared at 674.05 (m/z), which corresponds to $[\mathbf{1} + Pb^{2+} - H^+]^+$ and a new peak at 1135.15 was observed, which corresponds to $[2^*\mathbf{1} + Hg^{2+} - H^+]^+$.

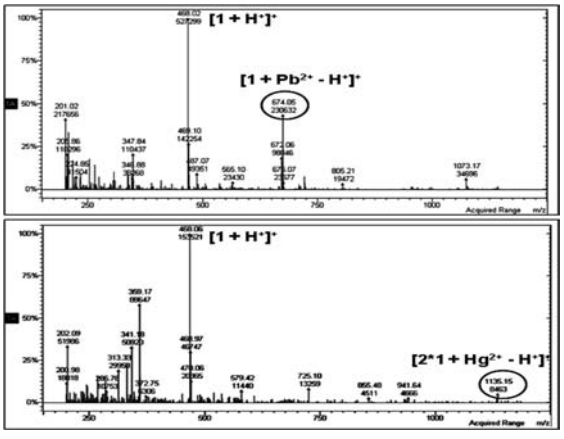

Figure 4. ESI mass spectra of **1** (500 μM) in the presence of Pb(II) or Hg(II) (1 equiv) in 30% CH_3CN/H_2O.

Overall results indicates that **1** forms a 2:1 complex with Hg(II) and a 1:1 complex with Pb(II), respectively. Using

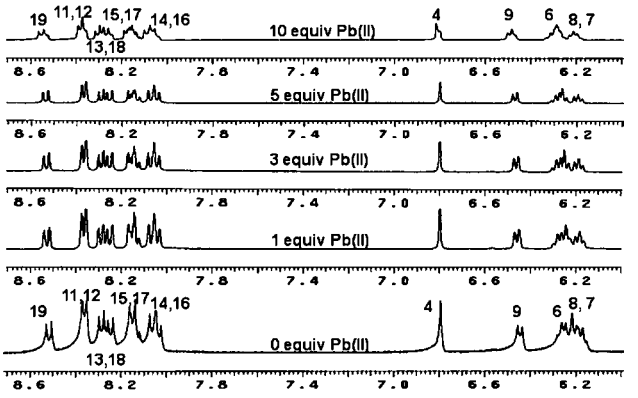

Figure 5. Partial ^{1}H NMR spectra of **1** (14 mM) in the presence of $Pb(ClO_4)_2$ in D_2O/DMSO-d_6 (1:2, v/v).

the fluorescence titration and stoichiometry data, the binding constant of **1** with Hg(II) and Pb(II) was found to be 1.12×10^{13} M^{-2} ($R^2 = 0.97$) and 3.11×10^2 M^{-1} ($R^2 = 0.99$) respectively (Figure S10).

The binding mode of **1** with the metal ion was further investigated by NMR spectroscopy (Figure 5). ^{1}H NMR titration experiments were carried out in D_2O−DMSO-d_6 because **1** showed a turn-on response to Pb(II) and Hg(II) in this solvent system. When the concentration of Pb(II) increased, the downfield shifts in H(9), H(6), and H(4) corresponding to the protons of indole ring were observed. The shifts of H(9) ($\Delta = 0.039$ ppm) and H(6) ($\Delta = 0.042$ ppm) may be attributed to the coordination between Pb(II) and the indole moiety possibly by cation−π interactions. The chelation between metal ions and Trp was reported in the metal recognition of metalloproteins.[20] The addition of Pb(II) induced a downfield shift of the pyrene protons between 8.0 and 8.6, which indicates that Pb(II) also interacted with the pyrene moiety. We also measured the ^{1}H NMR spectra of **1** in the presence of Hg(II) (Figure S11). The downfield shifts in H(9), H(6), and H(4) corresponding to the protons of indole moiety were observed with the gradual addition of Hg(II), which indicates the chelation of Hg(II) with the indole moiety. The considerable downfield shifts of H(19) in the presene of Hg(II) also suggests the chelation of Hg(II) with the pyrene moiety of **1**.

To confirm the quenching effect of the Trp amino acid on the pyrene fluorophore, we compared the quantum yields between pyrene labeled Gly (Pyr-Gly) that did not contain an indole ring and **1**. The quantum yields of Pyr-Gly and **1** are 0.85 and 0.0041, respectively, which indicates that the large fluorescent quenching is caused by the Trp moiety including the indole group. We also measured the quantum yields of **1** in the presence of Pb(II) and Hg(II), respectively. The quantum yields of the **1**−Pb and **1**−Hg complexes are 0.038 and 0.056, respectively, which reveals

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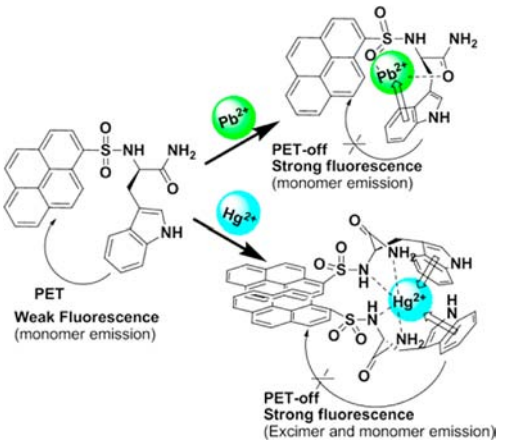

Figure 6. Proposed binding mode of **1** with Pb(II) and Hg(II).

that a large fluorescence enhancement was induced by the binding of Pb(II) or Hg(II).

The binding mode of **1** with the metal ions can be proposed by ^{1}H NMR titration experiments and the fluorescent spectroscopy data of **1** with Pb(II) and Hg(II) and the binding mode of the previously reported chemosensors based on the amino acid with metal ions (Figure 6).[12d,h,15a−,15c] The chelation of Pb(II) with the indole group of **1** by cation−π interactions inhibits PET from the indole group to the pyrene fluorophore, resulting in the increase of pyrene monomer emission. In the case of Hg(II) binding, two molecules of **1** interact with one Hg(II), resulting in pyrene excimer emission by pyrene−pyrene dimerization, and simultaneously, the chelation of Hg(II) with the indole groups inhibits PET from the indole group to the pyrene fluorophore, resulting in the increase of pyrene monomer emission as well as an increase of excimer emission.

In summary, we present a new fluorescent chemosensor for detecting Pb(II) or Hg(II) in aqueous solutions by a turn-on response and have first reported that Trp acts as a ligand as well as a quencher for recognition and fluorescent change by the metal binding event, which will provide information for the design of fluorescent chemosensors for metal ions and Trp-containing peptides for structural studies.

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Supporting Information Available. Synthesis, experimental details, and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.