ORGANIC LETTERS

2012
Vol. 14, No. 18
4746–4749

# Highly Sensitive Ratiometric Fluorescent Chemosensor for Silver Ion and Silver Nanoparticles in Aqueous Solution

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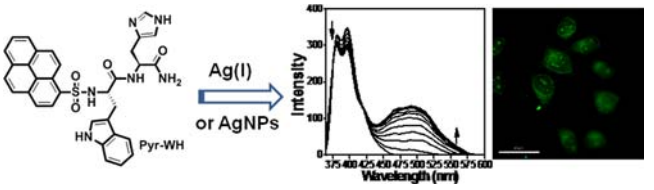
**Received July 18, 2012**

## ABSTRACT




**A pyrene derivative chemosensor (Pyr-WH) based on a dipeptide shows a highly sensitive ratiometric response to Ag(I) as well as silver nanoparticles in aqueous solution at physiological pH. Pyr-WH penetrated live HeLa cells and exhibits a ratiometric response to intracellular Ag(I). The binding mode of Pyr-WH with Ag(I) was characterized based on fluorescence changes in different pH, NMR, and ESI mass spectrometer experiments.**

There are many efforts devoted to the development of artifical receptors for sensing heavy and transition metal ions (HTMs) because these metal ions showed severe toxicity to living organisms including humans.[1,2] Silver ions have been regarded as nontoxic metal ions. However in recent years, bioaccumulation and the potential toxicity of Ag(I) and silver nanoparticles (AgNPs) to benign bacteria, amphibian, and fishes in waters have been reported.[3] Additionally, it is known that silver can inactivate sulphydryl enzymes and accumulate in the body.[3d] Thus, there are many developmental studies focused on fluorescence chemical sensors for Ag(I).[4] However, the number of reported chemosensors for Ag(I) are much less than those of chemosensors for other heavy metal ions. Almost all reported chemosensors for Ag(I) have some

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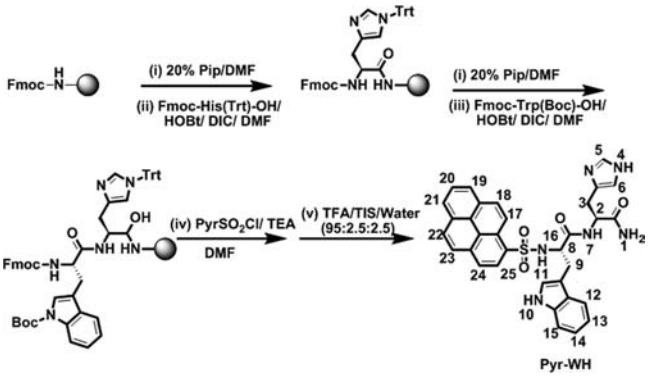
10.1021/ol301991h 
**Published on Web 09/05/2012**

limitations such as low water solubility, poor sensitivity, and low selectivity. As the quenching process can be caused by other external factors and Ag(I), the new fluorescent sensors for Ag(I) are highly recommended for turn-on or ratiometric responses. Ratiometric sensing is more ideal because this type of response makes it possible to measure analytes more accurately with minimization of background signal.[5] However, ratiometric sensors for Ag(I) have been rarely reported and none of the chemosensors for Ag(I) show a ratiometric response to AgNPs.[6,7] In addition, the reported ratiometric fluorescent sensors for Ag(I) required a high proportion of organic solvent in media for proper operation. Especially, to the best of our knowledge, none of them have been demonstrated to detect Ag(I) in live cells. Thus, it is highly challenging to synthesize ratiometric fluorescent sensors that detect Ag(I) and AgNPs in an aqueous solution.

Many metalloproteins were complexed with heavy and transition metal ions for various types of biological processes.[8] Amino acids in the metal binding sites of metalloproteins play a critical role in the protein−metal interactions. Especially, the side chain interaction of the amino acids in the metal binding sites for metal ions significantly affects the metal recognition of the proteins.[9] For example, a His residue containing an imidazole group was reported to interact with several heavy metal ions including Ag(I).[10] Recently, we and other research groups successfully synthesized fluorescent chemical sensors for HTMs based on amino acids and peptides in the metal binding sites of the proteins.[11,12]The fluorescent chemosensors based on amino acids showed high sensitivity and selectivity in aqueous solutions. However, the fluorescent chemosensors based on dipeptides have not been well investigated.

**Scheme 1.** Synthesis of **Pyr-WH**




As shown in Scheme 1, a pyrene labeled TrpHis (**Pyr-WH)** was synthesized in a solid-phase synthesis with a high yield (76%). Pyrene fluorophores have been frequently used for the synthesis of fluorescent chemical sensors because unique monomer and excimer emissions occur depending on the relative proximity between pyrene fluorophores.[13] Thus, we synthesized pyrenesulfonyl chloride and conjugated it to the *N*-terminal of the dipeptide in solid phase synthesis. Details on the synthesis and characterization of **Pyr-WH** are described in the Supporting Information (Figures S1−S5).

We investigated the metal ion binding properties of **Pyr-WH** in an aqueous solution at pH 7.4 by fluorescence change (Figure 1). The photochemical experiments were carried out in an aqueous solution containing 1% of DMF or in a 100% aqueous solution. **Pyr-WH** shows a sensitive ratiometric response to Ag(I) and Hg(II) among 14 metal ions in a buffer solution at pH 7.4 containing 1% of DMF (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).

In the absence of metal ions, typical monomer bands at 378 and 395 nm without an excimer peak at 480 nm were observed. The addition of Ag(I) to a solution of **Pyr-WH** led to a decrease in the monomer emission bands and a considerable increase of the excimer band at 480 nm, which indicates that two **Pyr-WH** interacted with Ag(I) and the

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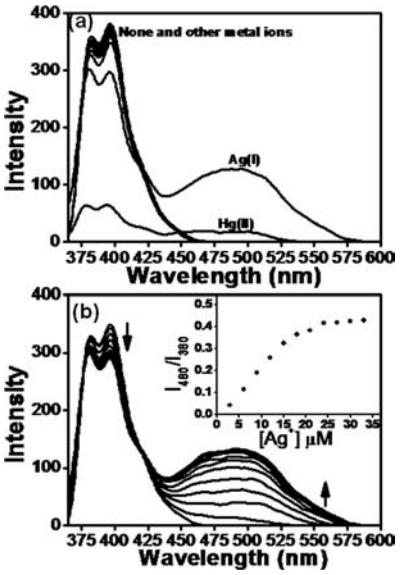


**Figure 1.** (a) Fluorescence response of **Pyr-WH** (30 $\mu$M) in the presence of various metal ions (1 equiv); (b) fluorescence changes of **Pyr-WH** (30 $\mu$M) in the presence of various concentrations of Ag(I) (0−1.0 equiv) in 10 mM HEPES buffer solution (pH 7.4, 1% DMF).

pyrene moieties of two chemosensors were stacked in the presence of Ag(I). Figure 1b shows the gradual emission intensity change of **Pyr-WH** upon addition of Ag(I). The intensity ratio ($I_{480}/I_{380}$) at 480 and 380 nm changed significantly from 0.0012 to 0.4358 (ca. 363 fold) by adding Ag(I) (Figure S6). A complete change in the emission intensity required ~1.0 equiv of Ag(I). The fluorescene response of **Pyr-WH** to Ag(I) was investigated in 100% aqueous solutions at pH 7.4 (Figure S7). A strong monomer emission and weak excimer emission of pyrene were observed even in the absence of Ag(I). This indicates that there are some interactions between the two pyrene moieties of **Pyr-WH** in a 100% aqueous solution. When Ag(I) was added into the solution of **Pyr-WH**, the excimer emission intensity increased, whereas the monomer emission intenisty decreased. The intensity ratio ($I_{480}/I_{380}$) changed from 0.11 to 0.73 (ca. 6.6-fold) by adding Ag(I). The ratiometric response of **Pyr-WH** to Ag(I) was much better in an aqueous solution containing 1% DMF rather than a 100% aqueous solution.

The sensor also showed the ratiometric response to Hg(II) in a buffer solution containing 1% DMF (Figure S8). Intertestingly, the chemosensor differentiated Ag(I) and Hg(II) by ratiometric response type. Upon Ag(I) binding, the sensor exhibited a strong excimer emission at 480 nm with a small decrease of monomer emission bands, whereas, upon Hg(II) binding, the sensor showed a weak excimer emission with a considerable decrease of the monomer emission bands. Job's plots that exhibited a maximum at a 0.33 mol fraction indicates that **Pyr-WH** formed a 2:1 complex with Ag(I) and Hg(II), respectively (Figure S9). Assuming a 2:1 complex formation, the association constants of **Pyr-WH** for Ag(I) and Hg(II) were calculated as $9.41 \times 10^{12}$ M$^{-2}$ ($R^2 = 0.96$) and $2.26 \times 10^{12}$ M$^{-2}$ ($R^2 = 0.93$), respectively.[14] In the UV/vis titration of Ag(I) and Hg(II), a significant decrease of the absorbance at 350 nm and a hypochromic shift of the absorption spectra were observed (Figure S10). This is in agreement with the formation of pyrene dimers in the ground state in the presence of Ag(I) or Hg(II).[14]

To investigate the role of each amino acid residue of the chemosensor for the interaction with Ag(I) and Hg(II), the emission spectra of **Pyr-WH** were measured at various pH values in the presence and absence of Ag(I) and Hg(II), respectively (Figure S11). This result indicates that the imidazole group ($pK_a \cong 6$) of the sensor plays a critical role in the interaction with Ag(I) because the sensor did not show any response to Ag(I) at pH = 4.5 and 5.5, whereas the sensor showed a sensitive ratiometric response to Ag(I) at neutral and basic pH. Interestingly, **Pyr-WH** showed a sensitive response to Hg(II) under acidic conditions, which strongly supports that the His residue plays a critical role in the interaction with Ag(I).

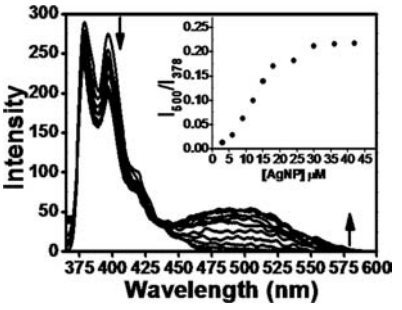


**Figure 2.** Fluorescence titration spectra of **Pyr-WH** (30 $\mu$M) in 10 mM HEPES buffer solution (pH 7.4, 1% DMF) by adding AgNPs (0−1.4 equiv).

We investigated whether **Pyr-WH** detected AgNPs by a ratometric response (Figue 2). According to the reported method,[4f,8] AgNPs were oxidized by $H_2O_2$, resulting in the equilibration of AgNPs to Ag(I). Thus, **Pyr-WH** was used to detect various concentrations of AgNPs. **Pyr-WH** showed a ratiometric response to the solution containing AgNPs. The intensity ratio at 500 and 378 nm changed significantly by adding AgNPs, which indicated that **Pyr-WH** is suitable for detecting AgNPs in an aqueous solution.

As **Pyr-WH** has a high binding affinity for Ag(I), we investigated the binding mode of **Pyr-WH** for Ag(I) by using organic spectroscopy techniques such as ESI mass spectrometry and NMR. When 1.0 equiv of Ag(I) was added to the **Pyr-WH** solution, a new peak appeared at 1317.07 ($m/z$), which corresponds to [2**Pyr-WH** + Ag$^+$]$^+$ (Figure 3). The mass spectrum also provides evidence of a 2:1 complex of **Pyr-WH** with Ag(I).

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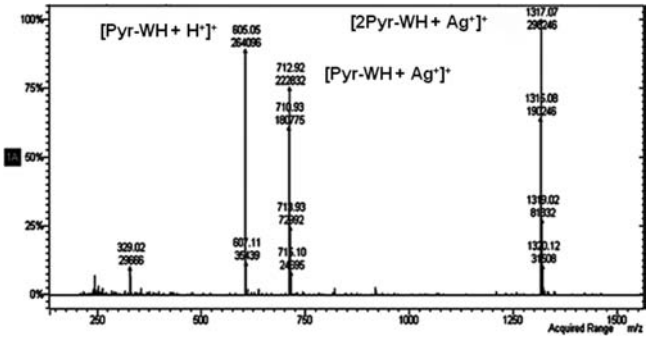


**Figure 3.** ESI mass spectrum of **Pyr-WH** (500 $\mu$M) in 50% $CH_3CN/H_2O$ including $AgClO_4$ (1 equiv).

The NMR study provides additional information about the binding mode of **Pyr-WH** by Ag(I) (Figure 4, Figure S12). When 1 equiv of Ag(I) was added, the disappearance of the chemical shifts in H(10) and H(11) was observed, which indicates that Ag(I) interacts with the moiety of Trp.

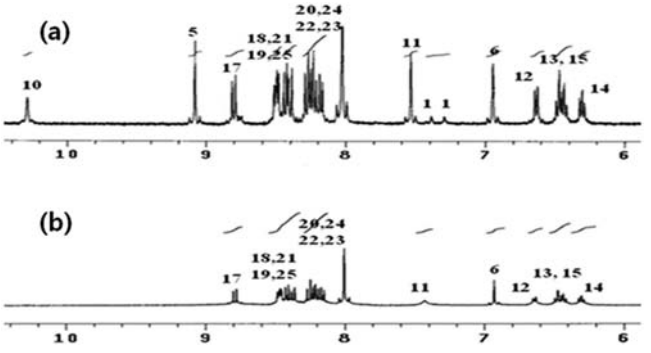


**Figure 4.** Partial $^1$H NMR spectra of **Pyr-WH** (5 mM) in the absence (a) and presence (b) of $AgClO_4$ (1 equiv) in $D_2O$/DMF (9:1, v/v) at 25 °C.

The disappearance of the chemical shifts in H(5) revealed that Ag(I) coordinated with the His residue of the peptide moiety. The NMR study revealed that Ag(I) coordinated with both His and the Trp residues of the chemosensor (Scheme 1). As the chemosensor exhibited a highly sensitive response to Ag(I) in aqueous solutions at physiological pH, we investigated whether the sensor penetrated live cells and detected intracellular Ag(I) by a ratiometric response. After we incubated **Pyr-WH** with HeLa cells for 1 h at 37 °C, the fluorescent change of the cells was monitored by microscopy (Figure 5). The blue color image of the cells reveals that **Pyr-WH** could penetrate HeLa cells under these conditions. **Pyr-WH** seems to possess sufficient cell penetration ability possibly due to the hydrophobic pyrene moiety. As a millimolar concentration of cellular chloride ions causes the precipitation of Ag(I) as AgCl, the HeLa cells were washed with a 20 mM HEPES buffer solution (pH 7.4) containing $NaNO_3$ instead of NaCl. After the addition of Ag(I) into the sensor loaded cells, a strong green color was observed in the cells, which indicated that **Pyr-WH** detected intracellular Ag(I) by a ratiometric response.

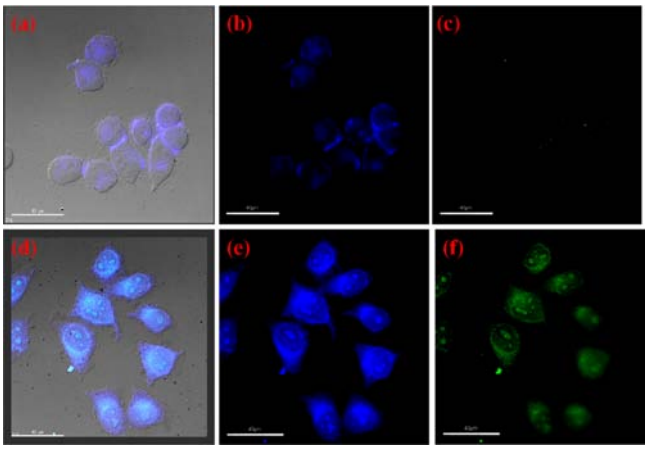


**Figure 5.** Fluorescence images of HeLa cells incubated with **Pyr-WH** (30 $\mu$M) (a–c) and HeLa cells incubated with **Pyr-WH** (30 $\mu$M) in the presence of $AgClO_4$ (2 equiv) (d–f). Bright field and fluorescence images (a, d), emission measured at 435 ± 48 nm (DAPI range) (b, e), emission measured at 523 ± 35 nm (GFP range) (c, f). The fluorescence images were observed by a DeltaVision microscope (Applied Precision).

In summary, we present a promising analytical approach for detecting Ag(I) and AgNPs in aqueous solutions. The chemosensor based on a dipeptide showed interesting properties such as high sensitivity for Ag(I) and AgNPs, a ratiometric response, and good water solubility. The chemosensor penetrated and detected intracellular Ag(I) in live cells by ratiometric responses.

**Acknowledgment.** This work was supported by a grant (2012R1AB3000574) from the Basic Research Program of the National Research Foundation and a grant (2012000540013) from the Korea Environmental Industry and Technology Institute.

**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.