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Ratiometric fluorescence detection of Hg(II) in aqueous solutions at physiological pH and live cells with a chemosensor based on tyrosine

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ABSTRACT

A fluorescent chemosensor (**Pyr-Tyr**) based on tyrosine showed highly selective and sensitive ratiometric response to Hg(II) among 14 metal ions in buffered aqueous solutions. The emission intensity ratio at 480 and 383 nm increased significantly from 0 to about 1.5 with the increase of Hg(II) concentrations (0–1 equiv.) and the ratiometric response to Hg(II) was not interfered by other metal ions such as Cu(II), Cd(II), and Ag(I). The detection limit of the chemosensor for Hg(II) was calculated to be 12 ± 1 nM (2.4 ppb). **Pyr-Tyr** exhibited ratiometric responses to Hg(II) in a wide range of pH values (pH 4.5–11.5) and the chemosensor was demonstrated to detect intracellular Hg(II) in HeLa cells. The binding mode of the chemosensor with Hg(II) was investigated by ^{1}H NMR titration and pH titration experiments, and ESI mass spectrometry.

1. Introduction

Methods for detecting heavy and transition metal ions have attracted considerable attention because these metal ions are toxic to humans and other living organisms [1,2]. In particular, the mercury(II) ion (Hg^{2+}), which is the most common oxidation form of mercury, contaminates aqueous solutions and accumulates in fish and crops, causing serious problems for human health and the environment [1,2]. Although atomic absorption spectrometry (AAS) and inductively coupled plasma-mass spectrometry (ICPMS) have been used for analysis owing to their high sensitivity, they require expensive instruments and time consuming procedures [3,4]. Alternatively, the use of fluorescent techniques for detecting Hg(II) is attractive due to their sensitivity, simplicity and low cost [5–8]. Therefore, many studies have been performed on the development of fluorescent chemosensors for Hg(II) with considerable progress [5–26]. On the other hand, most of chemosensors developed thus far have at least one drawback, such as low selectivity, interference from other metal ions, or poor solubility in aqueous solutions [5–26]. Moreover, new fluorescent chemosensors are preferred for a ratiometric response because the ratio between the two emission intensities can correct the sensor and analyte concentration as well as environmental effects, such as pH, polarity, photo-bleaching, and temperature [27,28]. In recent years, there have been much efforts to develop ratiometric chemosensors for Hg(II). However, very few chemosensors have successfully exhibited a sensitive ratiometric response to Hg(II) [29–40]. As the bioaccumulation of Hg(II) occurs mainly through water contamination [5–8], new ratiometric sensors are needed to operate in aqueous solutions. On the other hand, most ratiometric chemosensors for Hg(II) require a high proportion of organic solvents for their proper operations or showed low sensitivity in aqueous solutions, or interference with other heavy metal ions, such as Cu(II), Cd(II), and Ag(I) [29–40]. In addition, few ratiometric chemosensors have been applied to detect Hg(II) in biological samples [41–47]. However, most are not reversible chemosensors but reactive probes (chemodosimeters) [41–46]. Therefore, it is highly challenging to synthesize reversible ratiometric chemosensors that detect Hg(II) in aqueous solution as well as in live cells.

In recent years, fluorescent chemosensors based on hydrophilic biomolecules, such as amino acids, peptides, metalloproteins or DNA, have been reported to show hypersensitive responses to Hg(II) or Cu(II) in aqueous solutions because these biomolecules have hydrophilic and potent binding affinities to target metal ions in aqueous solutions [48–60]. Therefore, in this study, a reversible ratiometric chemosensor for Hg(II) was designed to mimic the metal binding site of metalloproteins. Among metalloproteins, mercuric reductase encoded by the mercury resistance operon,

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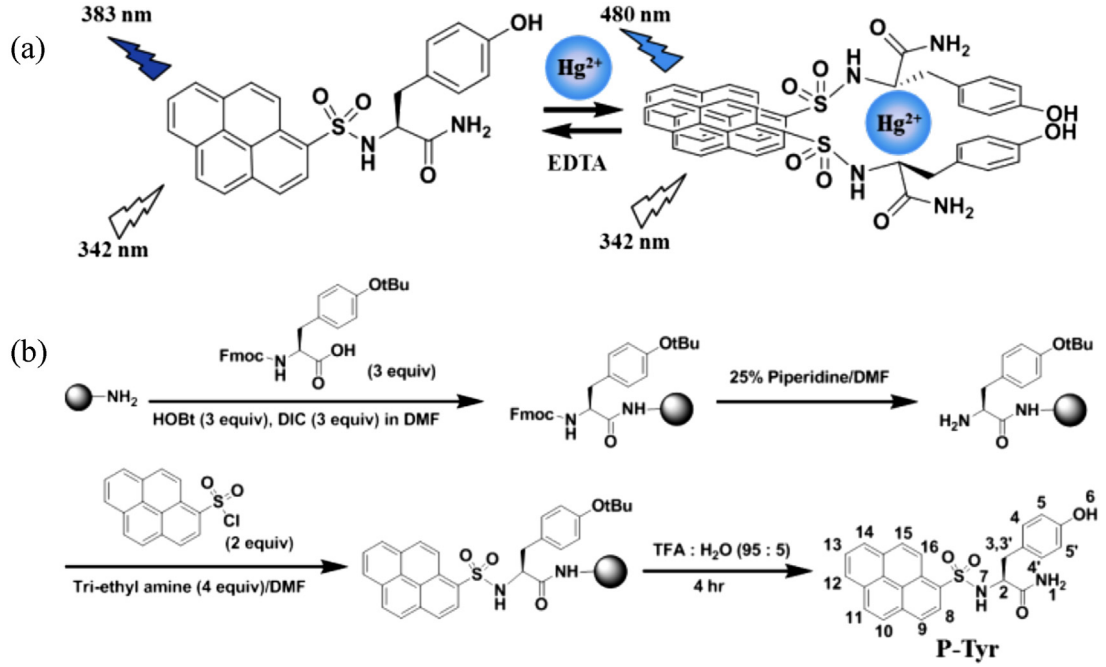

Scheme 1. (a) The proposed principle of **Pyr-Tyr** for detecting Hg(II). (b) Solid phase synthesis scheme of **Pyr-Tyr**.

catalyzes the reduction of Hg(II) to Hg(0) [61]. An X-ray crystallographic study reported that the Hg(II) binding site of mercuric reductase was formed by two Cys and two Tyr residues [62]. A Cys residue acts as a ligand for several heavy metal ions in some chemosensors [10], whereas a Tyr residue has not been used as a ligand in chemosensors. Therefore, in this study, we synthesized a fluorescent chemosensors (**Pyr-Tyr**) consisting of pyrene as the fluorophore and a Tyr residue as the receptor part to mimic half of the binding site (Scheme 1).

Pyrene was chosen as a fluorophore because 2:1 complexation of the chemosensor with Hg(II) might induce a change in ratio between the monomer and excimer emissions depending on the relative proximity between pyrene fluorophores [63]. The small fluorescent chemosensor employed in this study showed a selective and sensitive ratiometric response to Hg(II) in aqueous solutions and in HeLa cells.

2. Experimental

2.1. Reagents

Fmoc-Tyr(tBu)-OH, N,N-diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt), and Rink Amide MBHA resin were purchased from advanced Chem. Technol. Other reagents for solid phase synthesis including trifluoroacetic acid (TFA), triethylamine, N,N-dimethylformamide (DMF) and piperidine were purchased from Aldrich. 1-Pyrenesulfonyl chloride was synthesized from 1-pyrenesulfonic acid (purchased from Aldrich).

2.2. Solid phase peptide synthesis

Pyr-Tyr was synthesized in solid-phase synthesis with 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry (Scheme 1) [64]. DIC and HOBt in situ activation method was used for the coupling reactions. The amino acid Tyrosine with Fmoc as protecting group (0.3 mmol, 3 equiv.) was loaded to Rink Amide MBHA resin (0.1 mmol) according to the reported procedure. After washing, drying, and deprotecting the Fmoc group with 25% piperidine in DMF, 1-pyrenesulfonyl chloride (0.2 mmol, 2 equiv.) was coupled with the deprotected amino group in the presence of triethylamine (0.4 mmol, 4 equiv.). Similarly, 1-pyrenecarboxylic acid (0.2 mmol, 2 equiv.) was conjugated with the amino group of Tyrosine in the presence of DIC (0.3 mmol, 3 equiv.) and HOBt (0.3 mmol, 3 equiv.). After complete of the coupling reaction, the cleavage of **Pyr-Tyr** from the resin was accomplished with TFA/Water (95:5, v/v) at room temperature for 4 h. After removal of excess of TFA with N_2, crude product was precipitated by addition of cold ether into the cleavage solution. The precipitated crude product was centrifuged, washed with ether, and lyophilized under vacuum. The crude product was further purified with semi preparative HPLC using water (0.1% TFA)/acetonitrile (0.1% TFA) gradient to give 75% of **Pyr-Tyr**. The product was characterized by ^{1}H and ^{13}C NMR and ESI-mass data. **Pyr-Tyr**: White solid, mp 168–170 °C; ^{1}H NMR (400 MHz, 30% DMSO-d_6 in D_2O) δ 8.70 (d, J = 8.6 Hz, 1H), 8.56–8.50 (m, 1H), 8.42 (d, J = 8.4 Hz, 1H), 8.39 (d, J = 8.4 Hz, 1H), 8.35–8.27 (m, 3H), 6.57 (d, J = 8.0 Hz, 2H), 5.77 (d, J = 8.0 Hz, 2H), 3.99 dd, J = 3.5, 2.0 Hz, H), 2.92 (dd, J = 3.5, 2.0 Hz, 1H), 2.58 (dd, J = 8.0, 2.5 Hz, 1H); ^{13}C NMR (100 MHz, 30% DMSO-d_6 in D_2O) δ 172.9, 156.3, 134.5, 133.8, 131.2, 130.7, 130.5, 130.4, 129.7, 127.9, 127.7, 127.6, 127.4, 127.3, 127.1, 124.9, 124.6, 124.4, 123.9, 115.2, 58.6, 38.5; ESI-Mass (m/z): [**Pyr-Tyr**+H^+]$^+$ calcd for $C_{25}H_{21}O_4N_2S_1$: 445.11, obsd: 445.12.

2.3. Fluorescence measurements

A stock solution of **Pyr-Tyr** with the concentration of 2.00×10^{-3} M was prepared in DMF and stored in the dark place at 4 °C. Alternatively, a stock solution of **Pyr-Tyr** with the concentration of 0.50×10^{-3} M was prepared in distilled water containing 1 equiv. NaOH and stored in the dark place at 4 °C. This stock solution was used for fluorescence experiments after appropriate dilution. The fluorescence titration was carried out using the above referred solutions after maintaining the pH of the solution to 7.4 using 10 mM HEPES buffer solution. Fluorescence emission spectrum of a sample in a 10 mm path length quartz cuvette was measured in 10 mM HEPES buffer solution at pH 7.4 using a Perkin Elmer luminescence spectrophotometer (model LS 55). Emission spectra (360–600 nm) of **Pyr-Tyr** in the presence of metal ions (Na^+, K^+, and Al^{3+} as chloride anion and Ag^+, Cd^{2+}, Co^{2+}, Hg^{2+}, Cr^{3+}, Mg^{2+},

Ni^{2+}, Fe^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} as perchlorate anion) were measured by excitation with 342 nm. The slit widths for excitation and emission were 10 and 5 nm, respectively. The concentration of **Pyr-Tyr** was confirmed by UV absorbance at 352 nm for pyrene group. The association constants were calculated based on the titration curve of the sensor with metal ions. Association constants were determined by a nonlinear least square fit of the data with the following equation as referenced elsewhere [65]:

$$[\text{Hg(II)}] = \left\{ \frac{x}{2\text{¦Ka¦} \times \text{¦[Pyr-Tyr]} \times (1 - x^2)} \right\} + \frac{\boldsymbol{x} \times [\text{Pyr-Tyr}]}{2}$$

where $\boldsymbol{x}$ is $I - I_0/I_{\text{infinte}} - I_0$, I_0 is the intensity measured at zero concentration of Hg(II) and I_{infinte} is the intensity measured at infinite concentration of Hg(II).

The detection limit was calculated based on a fluorescence titration. To determine the S/N ratio, the emission intensity of free **Pyr-Tyr** was measured 10 times and the standard deviation of the blank measurements was determined. Three independent measurements of the emission intensity with a duplication were performed in the presence of Hg(II), and the average intensity was plotted as a concentration of metal ions to determine the slope. The detection limit was calculated using the following equation:

$$\text{Detection limit} = \frac{3\sigma}{m}$$

where σ is the standard deviation of the intensity of free sensor, m is the slope between the intensity at 384 nm vs concentration.

3. Results and discussion

Pyr-Tyr was synthesized in 75% yield using solid-phase synthesis. Details of the synthesis and characterization of **Pyr-Tyr** are described in the Supporting Information (Figs. S1–S4). The fluorescence property of **Pyr-Tyr** was dependent on the solvent systems. The fluorescence experiments were carried out in a 100% aqueous solution and aqueous solution containing DMF (1.5%). The emission spectra of free **Pyr-Tyr** itself showed typical pyrene monomer bands at 383 and 400 nm in aqueous solutions (Figs. 1 and S5).

The fluorescence spectra of **Pyr-Tyr** in the presence of various metal ions (1 equiv.) were measured to test the selectivity of **Pyr-Tyr** for metal ions. Interestingly, **Pyr-Tyr** showed a ratiometric response to Hg(II) among 14 metal ions by decreasing the monomeric emission bands and increasing the pyrene excimer band. Upon the addition of increasing amounts of Hg(II), a significant decrease in the monomer emission intensity at 383 nm as well as an increase in the pyrene excimer emission intensity at 480 nm was observed with a clear isoemissive point at 435 nm (Figs. 1b and 2).

In both solvent systems, a complete change in the emission intensity required approximately 1.0 equiv. of Hg(II), and a larger concentration of Hg(II) did not induce considerable changes in both the monomer emission intensity and excimer emission intensity. This suggests that **Pyr-Tyr** is hypersensitive to Hg(II) in aqueous solutions with and without DMF. Considering the excimer emission intensity, **Pyr-Tyr** shows an OFF–ON response to Hg(II). The emission intensity ratio (I_{480}/I_{383}) at 480 and 383 nm increased significantly from 0 to 1.5 with increasing Hg(II) concentration in aqueous solutions containing 1.5% DMF, whereas the ratiometric intensity ratio (I_{480}/I_{383}) changed from 0 to 0.74 in a 100% aqueous solution. The ratiometric response of **Pyr-Tyr** to Hg(II) appears to be better in aqueous solutions containing 1.5% DMF and it is more convenient in preparing stock solutions using DMF. Therefore, the photochemical properties of **Pyr-Tyr** for detecting Hg(II) ions were characterized further in aqueous solutions containing 1.5% DMF. We also investigate the effect of counter anions on the ratiometric response of **Pyr-Tyr** to Hg(II) ions. Similar ratiometric responses

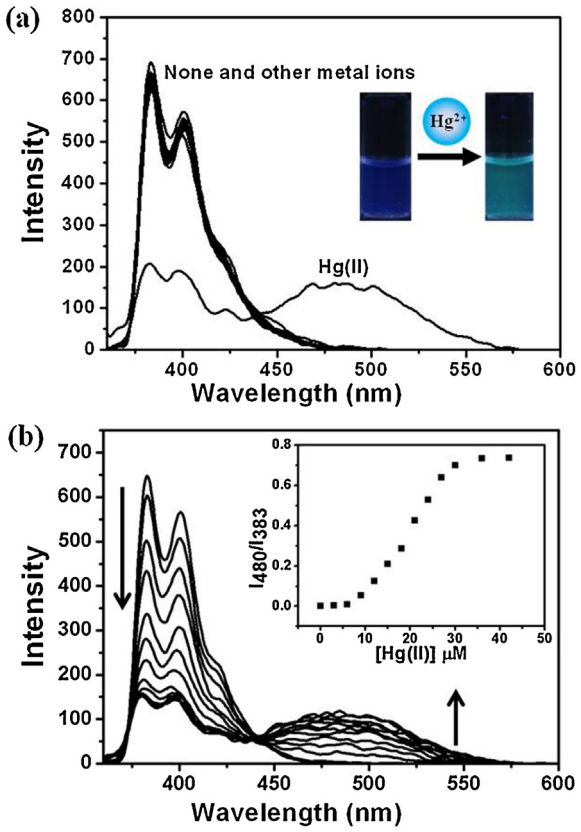

Fig. 1. (a) Fluorescence emission spectra of **Pyr-Tyr** (30 μM) in the presence of various metal ions (1 equiv.) in 100% aqueous solution (10 mM HEPES, pH 7.4). (b) Fluorescence spectra of **Pyr-Tyr** (30 μM) in 100% aqueous solution (10 mM HEPES, pH 7.4) adding Hg(II) (λ_{ex} = 342 nm).

were observed when different mercury salts such as $HgCl_2$ and $Hg(NO_3)_2$ were tested (data not shown). This reveals a little counter anion effect for detection of Hg(II) ions in this chemosensor.

The complexation of **Pyr-Tyr** to Hg(II) was examined using the UV/vis absorption spectrum (Fig. S6). Upon the addition of Hg(II), a significant decrease in the absorbance at 353 nm was observed, which was attributed to the intermolecular π–π stacked dimerization of the two pyrene moieties in the presence of Hg(II) [66]. The binding stoichiometry between **Pyr-Tyr** and Hg(II) was examined using a Job's plot. A Job's plot that exhibited a maximum at a 0.33 mol fraction suggests that **Pyr-Tyr** forms a 2:1 complex with Hg(II) (Fig. S7). The addition of Hg(II) induced 2:1 complexation

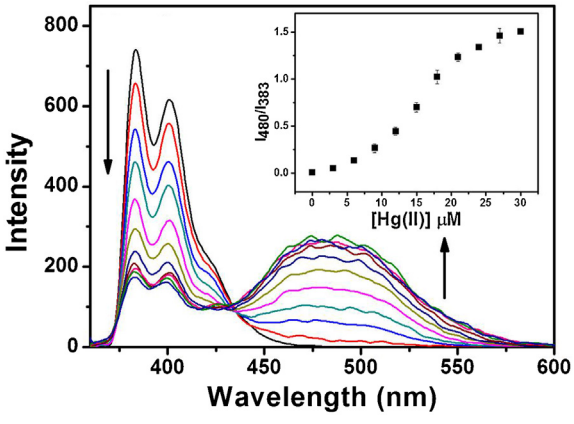

Fig. 2. Fluorescence spectra of **Pyr-Tyr** (30 μM) in 10 mM HEPES buffer solution at pH 7.4 containing 1.5% DMF adding Hg(II) (0–1.2 equiv.) (λ_{ex} = 342 nm).

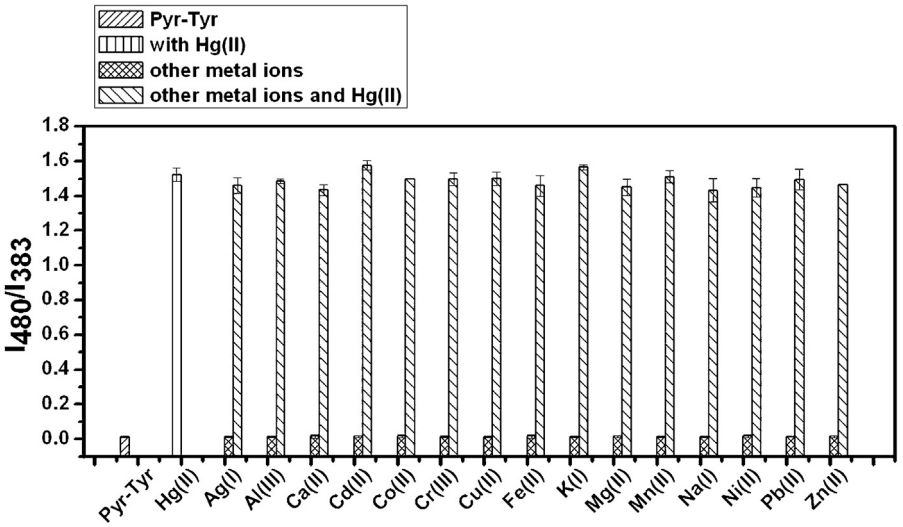

Fig. 3. Emission intensity of **Pyr-Tyr** (30 μM) in the presence of Hg(II) ions (1 equiv.) and additional metal ions (1 equiv.) in 10 mM HEPES buffer solution at pH 7.4 containing 1.5% DMF (λ_{ex} = 342 nm).

between **Pyr-Tyr** and Hg(II), resulting in an increase in excimer emission and a decrease in monomer emission. Assuming 2:1 complex formation, the association constant of **Pyr-Tyr** for Hg(II) was calculated to be $3.5 \times 10^{12}\ M^{-2}$ ($R^2 = 0.97$), which indicates that **Pyr-Tyr** has a significant binding affinity to Hg(II) in aqueous solutions (Fig. S8). The sensitivity of **Pyr-Tyr** was measured in the nanomolar concentrations (0–100 nM) of Hg(II). **Pyr-Tyr** showed a linear response of the emission intensity at 384 nm to the nanomolar concentrations of Hg(II) in aqueous solutions. The detection limit of 12 ± 1 nM (2.4 ppb) was calculated based on $3\sigma/m$, where σ is the standard deviation of the intensity of a free sensor, m is the slope of a plot of the intensity at 384 nm vs. concentration (Fig. S9). Although the detection limit of **Pyr-Tyr** was slightly higher than the maximum allowable level (10 nM, 2 ppb) of Hg(II) in drinking water demanded by the EPA, this value was much lower than those of most of the ratiometric sensors for Hg(II) previously reported [34–39].

To confirm the selectivity of **Pyr-Tyr** for Hg(II), competition experiments were performed in the presence of Hg(II) with other metal ions (Fig. 3). The ratiometric response of **Pyr-Tyr** to Hg(II) was unaffected significantly by other metal ions, such as Ag(I), Cu(II) and Cd(II), which are the most common competitive metal ions with Hg(II) [29–40]. This confirms that **Pyr-Tyr** has high selectivity for Hg(II) and can detect Hg(II) via a ratiometric response in the presence of other metal ions.

To test the reversibility, EDTA as a chelating agent for Hg(II) was added to a solution containing **Pyr-Tyr** and Hg(II) (1 equiv.), which showed a significant excimer emission intensity and a weak monomeric emission intensity (Fig. S10). The addition of EDTA to the solution resulted in an instant change in the excimer and monomer emission intensity. Approximately 1 equiv. of EDTA was sufficient to induce a return of the original metal free spectrum, which highlights the reversibility of **Pyr-Tyr**. The binding mode of **Pyr-Tyr** with Hg(II) was investigated using ESI mass spectrometry, ^{1}H NMR spectroscopy and pH titration experiments. The complex between **Pyr-Tyr** and Hg(II) was characterized by ESI mass spectrometry. **Pyr-Tyr** showed a ratiometric response to Hg(II) in 30% MeOH and H_2O. This solution containing **Pyr-Tyr** and Hg(II) was analyzed by ESI mass spectrometry (Fig. 4). When 1.0 equiv. of Hg(II) was added to the solution of **Pyr-Tyr**, a new peak appeared at 1088.32 (m/z), which corresponds to [2**Pyr-Tyr**+Hg^{2+}−H^+]$^+$. The ESI mass spectrum revealed the formation of a 2:1 complex and the tight binding affinity of **Pyr-Tyr** to Hg(II).

^{1}H NMR study provided a binding mode of **Pyr-Tyr** with Hg(II) (Figs. 5 and S11). We investigated whether **Pyr-Tyr** showed a ratiometric response to Hg(II) in H_2O/DMSO (7:3, v/v) to find a proper

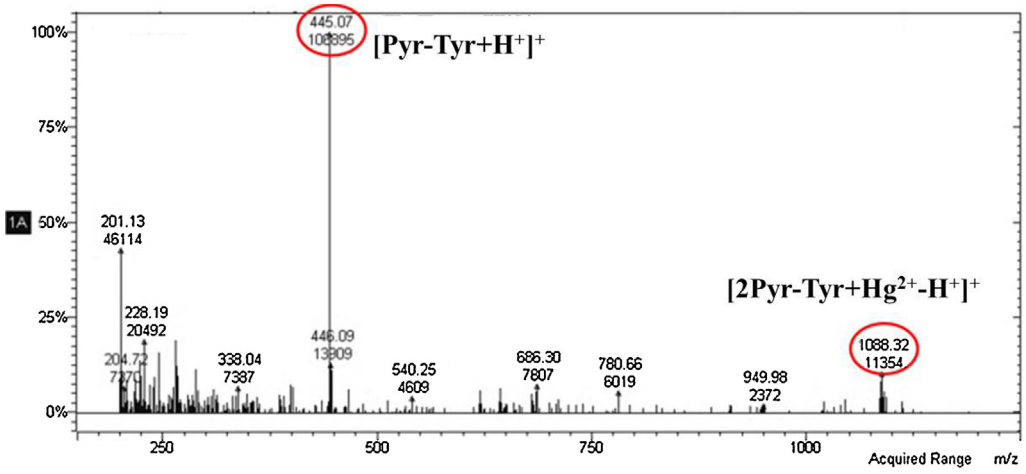

Fig. 4. ESI mass spectra of **Pyr-Tyr** in the presence of Hg(II) (1 equiv.) in 30% MeOH/H_2O.

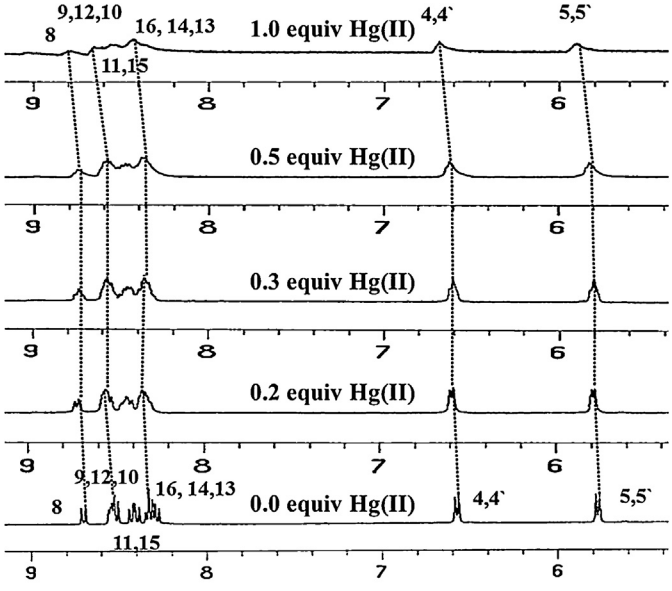

Fig. 5. Partial ^{1}H NMR spectra of **Pyr-Tyr** in the presence of $Hg(ClO_4)_2$ in D_2O/DMSO-d_6 (7:3, v/v).

solvent system for ^{1}H NMR titration experiments because the addition of Hg(II) into 100% aqueous solution containing 3 mM concentration of **Pyr-Tyr** instantly resulted in the precipitation. Even though the excimer intensity was much weak in H_2O/DMSO (7:3, v/v), **Pyr-Tyr** showed a ratiometric response to Hg(II) in this solvent condition (Fig. S12). Thus, ^{1}H NMR titration experiments were carried out in this solvent system. The addition of Hg(II) induced downfield shifts of the aromatic protons corresponding to the pyrene moiety. In addition, small down field shifts and broadening of the alpha and beta protons (H2, H3 and H3′) of the sulfonamide group were also observed, which suggest the interaction of the sulfonamide group with Hg(II). The considerable downfield shifts of aromatic protons (H4, H4′, H5, and H5′) of the phenol moiety were also observed, which might be due to coordination between Hg(II) and the phenol moiety of **Pyr-Tyr**. The result of NMR experiments suggests that the sulfonamide group and the phenol moiety of **Pyr-Tyr** play an important role in the binding of Hg(II) to the chemosensor.

The detailed binding mode of **Pyr-Tyr** with Hg(II) was proposed based on ^{1}H NMR titration experiments and the binding modes of the previously reported chemosensors for Hg(II) [34,47–50,67]. The sulfonamide groups in the previously reported chemosensors for Hg(II) acted as an important ligand for Hg(II) [34,47–50]. Specially, the X-ray crystal structure of the complex between dansyl-tryptophan methyl ester and Hg(II) revealed that the high stability of the complex between the chemosensor and Hg(II) could be attributed to the chelation of the nitrogen atom in the sulfonamide group and Hg(II) [67]. Thus, we could conclude that the sulfonamide group of **Pyr-Tyr** must be the critical ligand for the interaction with Hg(II). In the previous research, we reported that pyrene labeled Met was a ratiometric chemosensor for Hg(II) and confirmed that the combination of the binding sites involving the sulfonamide, amide carbonyl, and thioether groups of the chemosensor led to the strong binding to Hg(II) [34]. In comparison to the structure between pyrene labeled Met and **Pyr-Tyr**, the binding mode of the tyrosine moiety of **Pyr-Tyr** seems to be interesting because this chemosensor does not contain hydrazine or sulfur functional groups most commonly employed for Hg(II) binding. It is assumed that at least there are two possible binding mode of the tyrosine moiety for Hg(II). First, Hg(II) may interact with the OH group directly rather than the aromatic ring because the X-ray crystal structure of the complex between mercuric reductase and Hg(II) showed the direct interaction between the OH group of the Tyr and Hg(II) [62]. Alternatively, Hg(II) may interact with Tyr residue mainly by cation–pi interactions. In this case, the OH group may act as an electron donating group for increasing electron density of aromatic ring, resulting in the increase of the cation–pi interactions. Recently, the X-ray crystal structure of complex between dansyl-tryptophanmethyl ester and Hg(II) revealed that the weak interactions between indole aromatic ring and Hg(II) stabilized the complex [67]. In the previous reports, the coordination of Hg(II)

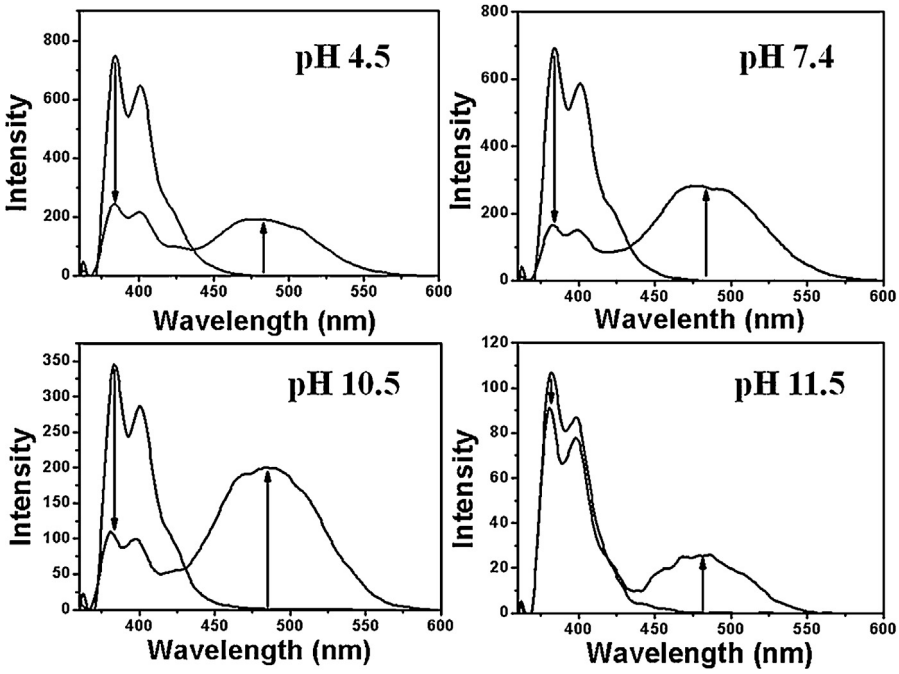

Fig. 6. The fluorescence response of **Pyr-Tyr** (30 μM) to Hg(II) (1 equiv.) in various pH.

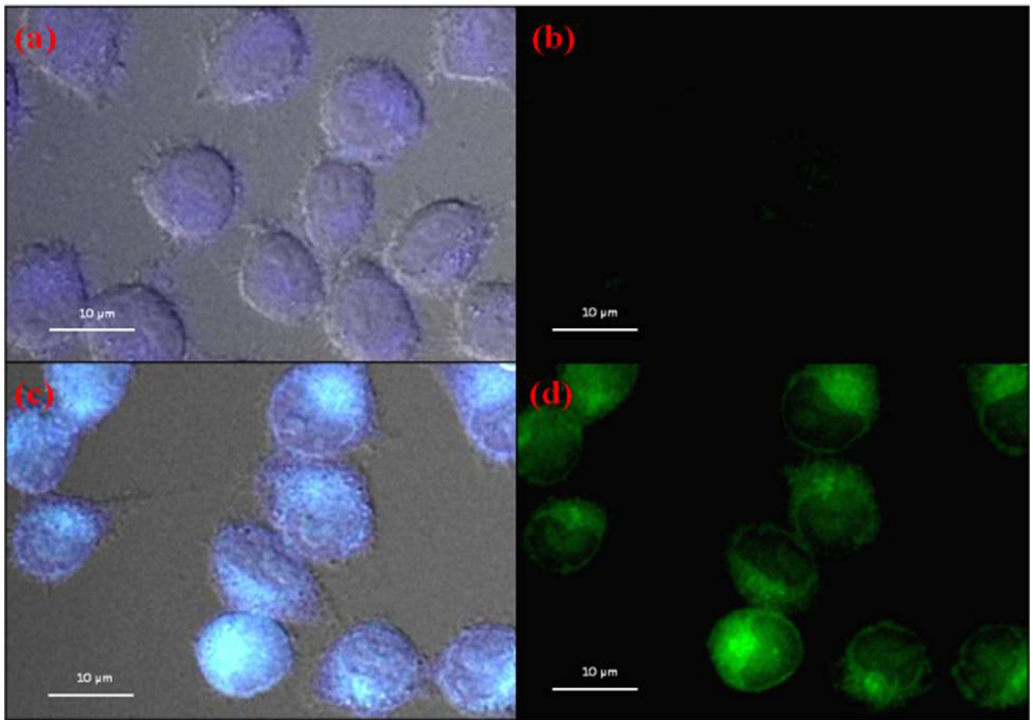

Fig. 7. Bright field and fluorescence images of HeLa cells incubated for 1 hr with **Pyr-Tyr** (30 μM) (a, b) and further incubated with $Hg(ClO_4)_2$ (2 equiv.) (c, d). Emission measured at 435 (± 48 nm) (left), emission measured at 523 (± 35 nm) (right).

and Tyr or aromatic rings were proposed by cation–pi interactions in X-ray structure and molecular modeling [68,69]. Thus, we assumed that Hg(II) bound to the tyrosine moiety of **Pyr-Tyr** by OH–Hg(II) interactions and/or cation–pi interactions. Considering the 2:1 complex of **Pyr-Tyr** with Hg(II), we could conclude that the combination of the binding sites of **Pyr-Tyr** involving the sulfonamide groups, amide carbonyl groups, and/or phenol groups played an important role for the complexation of the chemosensor with Hg(II).

To examine working pH and the role of the functional groups of **Pyr-Tyr**, emission spectra of **Pyr-Tyr** were measured in the presence and absence of Hg(II) in a range of pH (Fig. 6). In the acidic solution (pH = 4.5), **Pyr-Tyr** showed a ratiometric response to Hg(II). The emission intensity ratio (I_{480}/I_{383}) increased from 0 to 0.9 in the presence of 1 equiv. of Hg(II).

In general, the contaminated concentrations of heavy metal ions in water can increase in acidic pH due to the increase in solubility of heavy metal ions in acidic condition. On the other hand, most of the fluorescent chemosensors using PET or internal charge transfer (ICT) did not operate well under the acidic condition because the amine group in most chemosensors that played a critical role in the PET and ICT process was protonated under acidic conditions [5–1]. Under basic conditions (pH = 10.5 or 11.5), **Pyr-Tyr** maintained the ratiometric response to Hg(II). At pH = 10.5, the intensity of the monomeric emission bands was decreased more than that of the excimer emission intensity in the absence of Hg(II). Therefore, the emission intensity ratio (I_{480}/I_{383}) increased from 0 to 2.0 in the presence of 1 equiv. of Hg(II) at pH 10.5. The intensity of the monomeric emission band in the absence of Hg(II) was relatively weak at pH 11.5 which was attributed to quenching of the deprotonated sulfonamide group ($pK_a \approx 10$) and/or deprotonated hydroxyl group of the tyrosine ($pK_a \approx 10$) [70]. Nevertheless, **Pyr-Tyr** still showed a ratiometric response to Hg(II) at this pH. Interestingly, **Pyr-Tyr**, which recognizes Hg(II) by the signaling mechanisms of pyrene excimer formation, can maintain the ratiometric response to the target metal ion over the wide range of pH values (pH 4.5–11.5), whereas the ratiometric sensors using ICT did not maintain the ratiometric response to Hg(II) over the wide range of pH, mainly due to the protonation of amine group on the fluorophore at acidic pH.

As mercury ions in aqueous solution accumulate in living organisms, such as microorganisms, fish, and crops [4,5], a new mercury sensor that can detect Hg(II) in aqueous solution as well as biological samples is needed. Therefore, this study assessed the applications of **Pyr-Tyr** in biological samples, by examining whether **Pyr-Tyr** can penetrate live cells and detect intracellular Hg(II). After incubating the HeLa cells with **Pyr-Tyr** for 1 h at 37 °C, the blue color image of the cells was monitored by a Delta Vision microscopy (Applied Precision), as shown in Fig. 7.

Although **Pyr-Tyr** with a micromolar concentration dissolved in 100% aqueous solutions, **Pyr-Tyr** penetrated the lipid membranes of the HeLa cells, possibly due to the hydrophobic pyrene moiety [71]. After adding Hg(II) to the **Pyr-Tyr** loaded cells, a green color image was observed in the cells, which indicates an increase in the pyrene excimer intensity at approximately 480 nm due to an increase in intracellular Hg(II) levels within the living cells. Although **Pyr-Tyr** (30 μM) is soluble in a 100% aqueous solution and detects Hg(II) by 2:1 binding mode, this result suggests that **Pyr-Tyr** penetrates HeLa cells and properly operates for detecting intracellular Hg(II) through a ratiometric response.

4. Conclusion

We present a promising analytical approach for detecting Hg(II) in water without organic co-solvent and in live cells with a simple fluorescent chemosensor based on amino acid. The chemosensor based on a tyrosine residue showed remarkable properties for detecting Hg(II) such as a ratiometric response, high sensitivity and selectivity, no interference of other metal ions and good water solubility. The chemosensor was demonstrated to penetrate and detect intracellular Hg(II) by ratiometric response. The binding mode and simple structure of the sensor will provide information for the

design of new chemosensors for heavy metal ions that operates in aqueous solution and live cells.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2014.02.029.

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