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Ratiometric Determination of Hg^{2+} Ions Based on Simple Molecular Motifs of Pyrene and Dioxaoctanediamide

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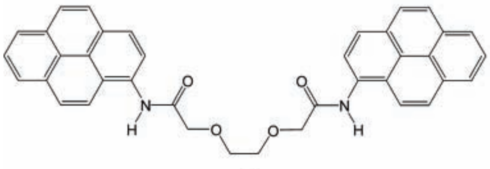
ABSTRACT



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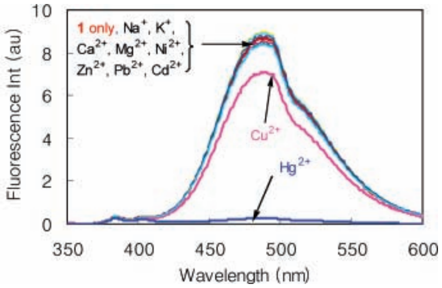

A new podand-type dioxaoctanediamide-based chemosensor having two pyrene moieties was prepared, and its fluoroionophoric behaviors toward transition-metal ions were investigated. Pyrene-appended dioxaoctanediamide 1 showed a selective fluorescence quenching toward Hg^{2+} ions over other transition-metal ions in an aqueous methanol solution. Unique responses in pyrene monomer and excimer emissions allowed selective ratiometric determination of Hg^{2+} ions in aqueous environments, and the detection limit was found to be 1.6×10^{-6} M.

A large number of functional ionophores having optical sensing ability have been continuously developed.[1] Particularly important in this field is the design of selective chemosensors for the analysis of heavy metal ions such as Hg^{2+}, Pb^{2+}, and Cd^{2+} due to their extremely toxic impact on our environment.[2] For the construction of efficient optical chemosensors, fluorescence is particularly attractive, and pyrene subunits are widely employed due to their well-known photophysical properties as well as their characteristic and environment-sensitive monomer or excimer emissions.[3] The introduction of two pyrene moieties, which can be situated closely enough to yield excimer emission, into an ion recognition system is a useful approach for the signaling of the complexation event by the changes in monomer and/or excimer emissions. Upon complexation with a specific guest ion, the conformations of the resulting compound could be fine-tuned to yield monomer and/or excimer emissions depending on the orientation of the two pyrene moieties.

Ratiometric measurements involve the observation of changes in the ratio of the intensities of absorption or

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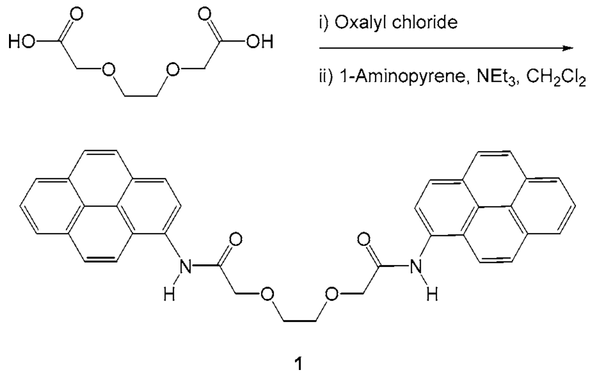
emission at two wavelengths. An important feature of ratiometric fluorescent probes is that they permit signal ratioing and, thus, increase the dynamic range while also providing built-in correction for environmental effects.[4] Consequently, ratiometric measurements are particularly attractive in the analysis of samples frequently encountered in real analytical systems which have a complex matrix.[5]

Metal ion-induced deprotonations of amides are interesting phenomena that have been utilized in the design of Cu^{2+} and Ni^{2+} selective chemosensors.[6] Recently, Hg^{2+} ion-induced deprotonation of the carboxamide was devised to signal the presence of Hg^{2+} ions in aqueous environments.[7] In this paper, we report the Hg^{2+}-selective chemosensing properties[8] of a simple chemosensor based on the dioxaoctanediamide molecular framework using the changes in the ratio of monomer and excimer emissions of pyrene fluorophore. The compound possesses an efficient Hg^{2+} selective ON−OFF-type signaling behavior that may be used for the ratiometric analysis of submillimolar Hg^{2+} ions in aqueous environments.

Diamide derivative **1** was prepared by the condensation of 3,6-dioxaoctanedioic acid with 1-aminopyrene in good yield (73%) (Scheme 1). 3,6-Dioxaoctanedioic acid molecular framework has been utilized as a platform for the construction of many efficient ionophores for the recognition of Pb^{2+}, Hg^{2+}, and Cd^{2+}.[9]

First, we attempted to determine the selective fluoroionophoric properties of **1** toward representative alkali (Na^+, K^+), alkaline earth (Mg^{2+}, Ca^{2+}), and transition-metal ions (Ni^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, Cd^{2+}). After systematically looking for selective signaling toward a specific target metal ion, we found that aqueous methanol solutions are relatively well-optimized sensing media. Therefore, all the fluorescence measurements were carried out in a 50% aqueous methanol solution ($MeOH/H_2O = 1:1$, v/v, $[\mathbf{1}] = 5.0 \times 10^{-6}$ M, $[M^{n+}] = 5.0 \times 10^{-4}$ M in HEPES buffer (10 mM, pH = 7.0)), where the most pronounced selectivity toward Hg^{2+} ions was realized (Figure S8, Supporting Information).

Scheme 1. Synthesis of Chemosensor **1**



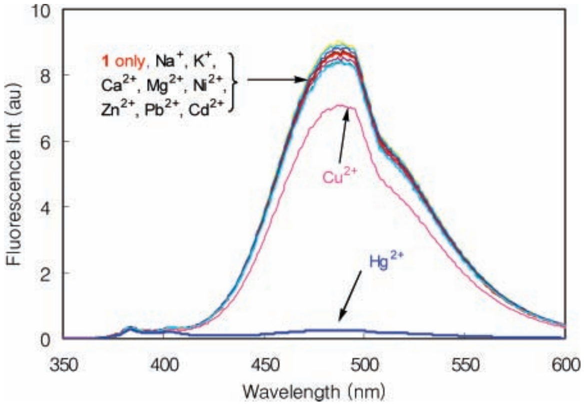


Figure 1. Fluoroionophoric properties of **1** in $MeOH-H_2O$ (1:1, v/v). $[\mathbf{1}] = 5.0 \times 10^{-6}$ M, $[M^{n+}] = 5.0 \times 10^{-4}$ M in HEPES buffer (10 mM, pH = 7.0).

As can be seen from Figure 1, the compound **1** exhibited an intense excimer emission of the pyrene centered around 489 nm with characteristic, but very weak, monomer emissions around 380−410 nm. The ratio of fluorescence intensity of the excimer and monomer observed at 489 and 383 nm, respectively, was very large and equal to 24.9 in the 50% aqueous methanol solution. This observation implies that the compound exists in mainly stacked or folded conformations with the two pyrene moieties situated closely enough to yield the excimer.

Upon interaction with 100 equiv of various metal ions (as perchlorate salts), Hg^{2+} ions alone exhibited effective fluorescence quenching among the tested metal ions. The fluorescence was quenched remarkably and the intensity at 489 nm was dramatically reduced in the presence of 100

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equiv of Hg^{2+} ions ($I/I_0 = 0.03$, where I and I_0 represent the fluorescence intensity in the presence and absence of metal ions, respectively). Other alkali, alkaline earth, and transition-metal ions produced insignificant responses where I/I_0 ranged from 0.96 to 1.03. Under the employed conditions, Cu^{2+} ions also induced a somewhat quenched fluorescence intensity ($I/I_0 = 0.81$) and appeared to interfere significantly with the Hg^{2+}-selective responses of **1**. On the other hand, the pyrene monomer emission of **1** around 380−410 nm was not significantly affected by the interaction with surveyed metal ions (I/I_0 measured at 383 nm = 0.80 (Cu^{2+}), 0.77 (Hg^{2+}), 1.03−0.95 (the other metal ions)).

As discussed above, compound **1** also exhibited significant responses toward Cu^{2+} ions. However, the selective determination of Hg^{2+} ions could be realized by a ratiometric approach. That is, the fluorescence intensity ratios for the excimer and monomer emissions of **1**, which were measured at 489 and 383 nm, respectively, in the absence and the presence of surveyed metal ions were almost constant (25.0 ± 0.18, Figure 2). Only Hg^{2+} ions induced a dramatic reduction (25-fold) in this ratio, which suggests that efficient discrimination of the response toward Hg^{2+} ions from the possible interference of Cu^{2+} ions is possible.[10]

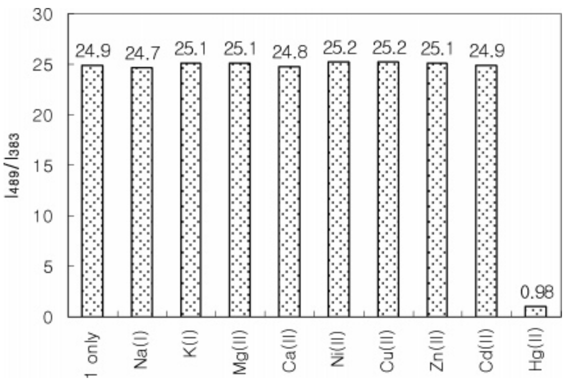

Figure 2. Ratios of excimer and monomer emissions (I_{489}/I_{383}) of **1** in the presence of varying metal ions. [**1**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M in HEPES buffered (10 mM, pH = 7.0) MeOH−H_2O (1:1, v/v).

The stoichiometry of the **1**−Hg^{2+} complex was estimated to be 1:1 by a nonlinear curve fitting of the fluorescence titration results and ^1H NMR titrations. As shown in the ^1H NMR spectra of Figure 3, treatment of Hg^{2+} ions resulted in significant shifts in the resonances of the dioxaoctanediamide backbone and pyrene moieties of **1**. The disappearance of the amide proton peak at 10.1 ppm was also interesting. The chemical shift changes were exclusively induced by Hg^{2+} ions while almost no changes were observed with other guests such as Na^+ and Ca^{2+} ions (Figure S11, Supporting Information). Particularly, a large shift in the C-1 signal from 131.5 to 142.2 ppm was observed in the ^{13}C NMR spectrum of compound **1** upon treatment with 2 equiv of $Hg(OAc)_2$ in DMSO-d_6 (Figure 3 (c and d)).[11] That might be due to the Hg^{2+}-induced deprotonation of the amide N−H groups of **1**.[7]

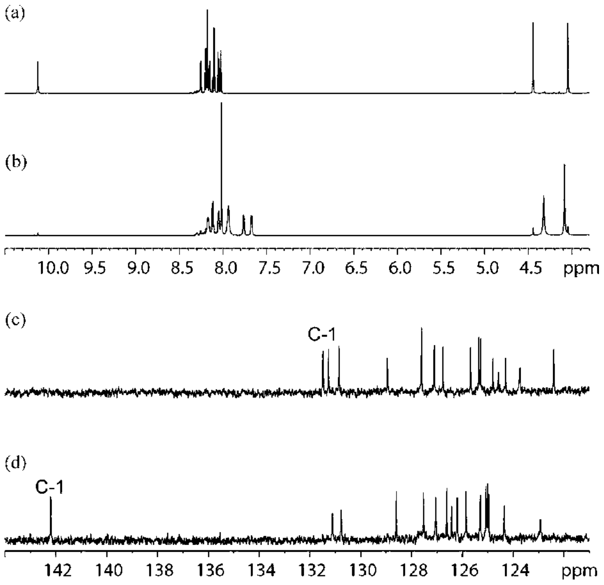

Figure 3. ^1H and ^{13}C NMR spectra of **1** only (a, c) and in the presence of Hg^{2+} ions (b, d) in DMSO-d_6. All spectra were obtained at 320 K for clarity.

The selective ON−OFF type of signaling behavior of **1** is believed to be due to the divergent orientation of the two pyrene moieties by the Hg^{2+}-induced selective deprotonation of the amide function as well as the intrinsic quenching nature of the complexed Hg^{2+} ions.[12] The disappearance of the excimer emission suggests that the complexed ligand would adopt a relatively extended conformation with divergent orientation of the two pyrene moieties. A similar observation was observed with Ca^{2+}-selective sensors based on the closely related structure having two pyrenes at both terminals of polyoxyethylene noncyclic crown ethers.[13] In that case, upon the complexation with alkaline earth metal ions, the sensors revealed an increase of monomer emission at around 400 nm accompanied by the disappearance of intramolecular excimer emission of free compounds. However, in the present system, the monomer region was also quenched by the complexed Hg^{2+} ions due to its intrinsic quenching nature.

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(10) The fluorescence intensity of monomer is rather weak compared with excimer emission for the ratiometric analysis of Hg^{2+} ions. We tried to find more optimized condition to have comparable fluorescence intensities at both monomer and excimer regions by changing water content and buffers. As the water content decreased, for example at MeOH−H_2O (9:1, v/v), the fluorescence intensity of monomer and excimer became somewhat balanced. However, the selectivity toward Hg^{2+} ions was greatly hampered (Figure S10, Supporting Information). The use of other buffers such as Tris (pH 8.1) and acetate (pH 4.8) also resulted in much reduced selectivity toward Hg^{2+} ions.

(11) The assignment of the C-1 resonance was made by COSY and HMBC experiments.

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The FAB-mass spectrum (*m*-NBA matrix) of **1**, measured upon treatment with 5 equiv of $Hg(OAc)_2$, revealed a prominent peak for the **1**$-Hg^{2+}$ complex at $m/z = 576.2$, which is ascribable to the amide deprotonated species of [**1** $- 2H + Hg]^+$. The deprotonation of amide functions by Hg^{2+} ions is previously reported in the Hg^{2+}-selective chemosensors derived from *o*-phenylenediamine based tri- or tetraamide.[7] IR measurements also suggest that the compound **1** complexes Hg^{2+} ions effectively by producing a significant shift in the absorption band of the amide carbonyl group from 1678 to 1616 cm^{-1}.

To gain more insight into the chemosensing properties of **1** toward Hg^{2+} ions, a fluorescence titration was carried out. The chemosensor exhibited very efficient fluorescence quenching, and over 70% of the total fluorescence intensity change was observed with 10 equiv of Hg^{2+} ions. The association constant, K_{assoc}, was estimated by a nonlinear least-square fitting[14] of the titration results and was found to be 6.3×10^4 M^{-1}. The detection limit[15] was also estimated from the titration results and was 1.6×10^{-6} M.

Finally, the practical applicability of the ionophore for the analysis of Hg^{2+} ions in the presence of physiologically important metal ions was investigated. The fluorescence titration of **1** with Hg^{2+} ions was carried out in the presence of physiologically important metal ions as background,[16] and the resulting profile was almost identical with that obtained in the absence of any metal ions (Figure 4). This observation confirms that the compound **1** may be used as a selective ratiometric chemosensor for the analysis of Hg^{2+} ions in the presence of other common competing metal ions.

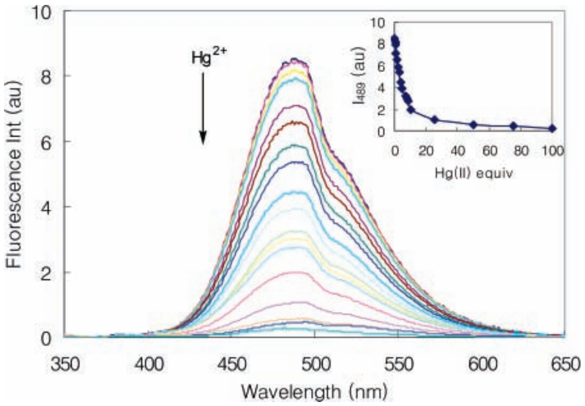

Figure 4. Fluorescence titration of **1** with $Hg(ClO_4)_2$ in the presence of physiologically important metal ions in MeOH$-H_2O$ (1:1, v/v). [**1**] $= 5.0 \times 10^{-6}$ M in HEPES buffer (10 mM, pH = 7.0). $[Na^+] = 138$ mM, $[K^+] = 4$ mM, $[Mg^{2+}] = 1$ mM, $[Ca^{2+}] = 3$ mM, $[Zn^{2+}] = 0.02$ mM, $[Cu^{2+}] = 0.015$ mM, and $[Co^{2+}] = 0.002$ mM.

In summary, we have prepared a new simple pyrenylamide derivative based upon the dioxaoctanediamide framework and investigated its chemosensing properties toward Hg^{2+} ions. The amide derivative exhibited a pronounced ON$-$OFF-type Hg^{2+}-selective fluorescence quenching behavior. The possible interference of significantly responding Cu^{2+} ions could be effectively circumvented by the ratiometric approach utilizing the changes in fluorescence intensity of the monomer and excimer region of pyrene fluorophore.

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Supporting Information Available: Experimental details and characterization for new compounds, NMR spectra, and fluorescence data are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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