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A Ratiometric Fluorescent Sensor for Ag^I with High Selectivity and Sensitivity

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The design and synthesis of fluorescent sensors with high selectivity and sensitivity for heavy and transition metal ions continues to grow at an unabated pace.[1] Regardless of the signal transducing function, signaling changes of a fluorescent molecule at two bands that exhibit enhancement of one band at the expense of the other upon cation binding, a ratiometric measurement can be made which can increase the selectivity and sensitivity of a measurement and can eliminate most or all of the possible variability due to differences in instrumental efficiency and content of effective dye.[2] Many investigations have been conducted to make ratiometric fluorescent sensors for metals;[3] however, few reports have been explored for Ag^I.[4] As many heavy cations are known as fluorescence quenchers, discrimination between Ag^I and chemically close ions presents a challenge.

We are interested in studying macrocyclic ligands with a fluorophore for use as selective metal ion chemosensors.[5] Herein, we report an effective ratiometric fluorescent sensor for Ag^I based on a pyrene-functionalized heterocycle receptor. The structures of fluorescent sensor **1** and analogue compound **2** are shown in Figure 1. Sensor **1** forms an intramolecular sandwich complex via silver ion-induced self-assembly that results in a dramatic increase in fluorescence intensity of the excimer and a dramatic decrease of monomer fluorescence and provides excellent selectivity for Ag^I over other heavy and transition metal ions.

Pyrene was selected as a signal-transducing unit. The emission wavelength of pyrene has been proven to be extremely sensitive to the polarity of the local environment. Formation of the self-assembled complex results in a remarkable change in fluorescence emission intensities of the pyrene excimer and monomer.[6] Thus, it is expected for the self-assembly of a pyrene-tethered receptor by specific binding to offer a novel approach to the sensing of substrates in solution.[7] To obtain the specific binding to Ag^I, a N,O-containing heterocycle was selected as the ion receptor unit. It was found that soft coordination sites seem to generate great affinity toward the d^{10} transition-metal ions such as Ag^I or Hg^{II}.[4] Compound **1** was synthesized in 45% yield by reacting prop-1-ene-1,3-sultone[5] with the corresponding nitrone in refluxing toluene for 24−48 h. To address the role the hydroxyl group played in the metal binding, compound **2** was also prepared by a similar procedure. The two compounds were obtained as racemic mixtures.

The fluorescence behavior of **1** was dependent on solvent and pH. The emission spectra of **1** and its fluorescence titration with metal ions were recorded in ethanol−water solution (50:50, v/v) at pH 7.0, the results of which are shown in Figure 2. The spectrum of the free ligand **1** shows typical emission bands at 378 and 397 nm (excitation 344 nm), which are attributed to the pyrene monomeric emission.[8] When Ag^I was added to the solution of **1**, a significant decrease of the pyrene monomer emission and a red-shifted, structureless maximum centered around 462 nm, typical of pyrene excimer fluorescence,[6] were observed with a clear isoemission point at 412 nm. The emission intensity ratio, I_{462}/I_{378}, increases with the increase in Ag^I concentrations, which allows free Ag^I concentration to be determined. It was noted that at the Ag^I concentration over 0.4 equiv relative to **1** concentration, the pyrene excimer emission had little deviation of I_{462}/I_{378}, which might be closely related to the formation of intramolecular excimer.[9] More Ag^I concentration results in a decrease in both the monomeric and the excimer emission intensities.

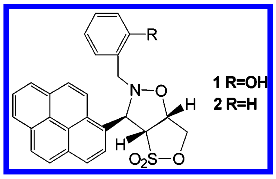

Figure 1. Drawing of fluorescent sensor **1** and analogue compound **2**.

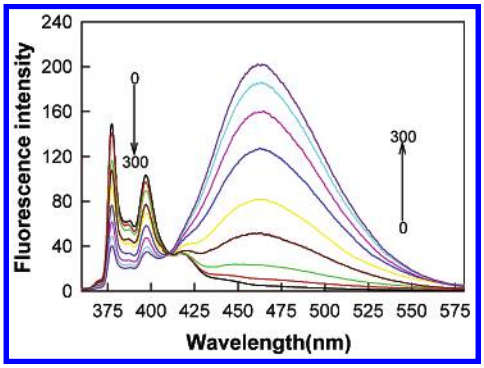

Figure 2. Fluorescence emission spectra of **1** in the presence of different concentrations of Ag^I (0, 4.0, 10, 15, 20, 40, 75, 150, 300 μM). Excitation wavelength was 344 nm. The concentration of **1** was 0.8 mM.

The fluorescence titration of **1** with various metal ions was conducted to examine the selectivity. Reaction of **1** with Ag^I and Hg^{II} leads to formation of pyrene excimer.[10] Among other ions investigated, alkali, alkaline earth ions, and anions have hardly an effect on **1** fluorescence, while transition metal ions (Cu^I, Cu^{II}, Pb^{II}, Mn^{II}, Co^{II}, Cd^{II}, Zn^{II}, Fe^{III}, and Hg_2^{II}) show strong quenching of monomer fluorescence; however, there is not any enhancement of the excimer emission of **1**. The quenching of monomer pyrene fluorescence results from the cation-π interactions between the heavy atom and the electron-rich aromatic.[11] Figure 3 shows the dependence of the intensity ratios of pyrene at 462 nm to that at 378 nm (I_{462}/I_{378}) on the cations; it can be easily recognized that high selectivity for Ag^I is obtained by compound **1**.

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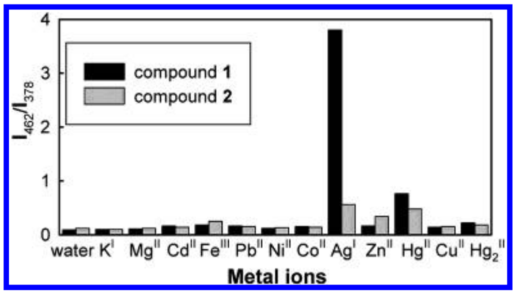

Figure 3. The responses of compounds **1** (black) and **2** (gray) to metal solutions (150 μM, *x*-axis markers). Excitation was at 344 nm, and emission was at 462 and 378 nm.

From the changes observed in the fluorescence intensity ratio, we were able to determine the stoichiometry and association constant of **1** with metal ions by the following equations:[12]

$$[\mathrm{M}^{n+}]^m = \frac{1}{n\cdot K}\cdot\frac{1}{[\mathrm{L}]_\mathrm{T}^{n-1}}\cdot\frac{1-\alpha}{\alpha^n} \tag{1}$$

$$\frac{1-\alpha}{\alpha} = n\cdot\frac{R-R_{\mathrm{min}}}{R_{\mathrm{max}}-R}\cdot\frac{\Phi_\mathrm{L}^{\lambda_2}}{\Phi_{\mathrm{M}_m\mathrm{L}_n}^{\lambda_2}} \tag{2}$$

where R is the fluorescence ratio of the ligand at λ_1 and λ_2, and R_{min} and R_{max} are the limiting values of R at zero cation concentration and at saturating cation concentration, respectively. Nonlinear fitting with α as a function of $\mathrm{Ag^I}$ concentration shows the formation of a 1:2 metal–ligand complex and gives a corresponding association constant for $\mathrm{Ag^I}$ of 2.2×10^5 M^{-2}.

To explore the effects of ethanol on the sensing behavior of **1** to metal ions, fluorescence responses of **1** to $\mathrm{Ag^I}$ were examined in ethanol/water with different volume ratios. In the ethanol percentages between 20 and 60, **1** exhibits strong and selective dual fluorescence responses of monomer and excimer to $\mathrm{Ag^I}$. As the ethanol percentage decreases, **1** exhibits one emission band at 450 nm. On the other hand, when the ethanol percentage exceeded 70, **1** exhibited a strong monomer emission; however, there was no excimer emission formation even at large $\mathrm{Ag^I}$ concentration. At 10% and 80% ethanol/water, the association constants of **1** with $\mathrm{Ag^I}$ from the titration data were calculated to be 4.2×10^4 and 6.8×10^3 M^{-2}, respectively.

Subsequently, fluorescence decay measurements manifest the interaction between **1** and $\mathrm{Ag^I}$. Fluorescence decay curves for **1** and its $\mathrm{Ag^I}$ compound were recorded at 378 and 462 nm. In the absence of $\mathrm{Ag^I}$, the decay times of the pyrene monomer and excimer were 11.6 and 14.4 ns, respectively. Moreover, the excimer fluorescence showed a rising time of 3.4 ns due to the formation of intramolecular excimer. By contrast, the excimer fluorescence from the silver compound showed a longer decay time (16.2 ns) and no rising time, which is in agreement with the observed binding-enhancement fluorescence. Therefore, the fluorescence from the silver compound is ascribed to the intramolecular excimer, which can be formed rapidly by the association between adjacent pyrene moieties in the molecule.

To gain insight into the role of the hydroxyl group of **1** in metal binding, compound **2** was also investigated. The results showed that upon reaction with $\mathrm{Ag^I}$, **2** displayed a fluorescence decrease both at 378 and at 398 nm with a little increase at 462 nm, indicating there was no formation of pyrene excimer. On the basis of these results, it is concluded that the fluorescence response of the sensor results from the formation of 2:1 complex with the metal ion, and the structureless broad band observed at 462 nm can be assigned to the emission of intramolecular excimer in the self-assembly of **1**, where binding of the metal center occurs through the phenolate hydroxyl group and the nitrogen atom of the heterocycle as schematically illustrated in Figure 4, in which the molecular modeling structure was energy-minimized using Chemoffice 7.0 MM2 utilities.

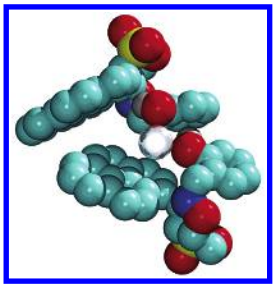

Figure 4. A proposed structure for compound **1** + metal ion.

In summary, we have developed a new fluorescent sensor for $\mathrm{Ag^I}$ with remarkably high selectivity and sensitivity. Moreover, this molecule makes it possible to detect the $\mathrm{Ag^I}$ cation ratiometrically, thereby eliminating most or all of the possible variability due to differences in instrumental efficiency and content of effective dye.

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Supporting Information Available: Synthesis and characteristics of **1** and **2**, sample preparation for fluorescence measurement, effects of $\mathrm{Hg^{II}}$ concentration on **1** emission and those of $\mathrm{Ag^I}$ concentration on **2** emission, nonlinear fitting curves for the stoichiometry, and the effects of coexistence on the $\mathrm{Ag^I}$ measurement (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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