Inorg. Chem. **2008**, *47*, 3946−3948

Inorganic Chemistry
Communication

Rosamine-Based Fluorescent Chemosensor for Selective Detection of Silver(I) in an Aqueous Solution

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Received March 11, 2008

The synthesis and photophysical properties of a rosamine-based fluorescent chemosensor, RosAg, for detecting Ag ion in an aqueous solution are described. This fluorescent sensor has a negligible quantum yield (<0.005) in the absence of Ag^+, whereas a significant increase in fluorescence is observed upon complexation with Ag^+ under physiological conditions. The crystal structure of the silver complex with the chelator moiety of RosAg reveals a trigonal-planar coordination geometry in which three S atoms occupy the metal center. Although a strong coordinative interaction of Ag−N is not observed in the crystal structure, the ^{1}H NMR experiments suggest that aniline nitrogen is likely to be associated with the Ag^+ center in the solution state. This may inhibit the photoinduced electron transfer process and result in the enhancement of fluorescence.

Selective and sensitive fluorescent sensors for metal ions have been essential tools not only in the field of biology but also in clinical and environmental studies.[1,2] In particular, these fluorescent sensors have been used in bioinorganic chemistry to understand the effects of metal ions, which often have important functions or produce toxic effects in cells, on the human body.[2,3] Among such biologically important metal ions, the silver ion (Ag^+) has long received considerable attention because of its antimicrobial activities.[4] Although several possible roles of Ag^+ in biological systems have been proposed, such as (i) interaction and inactivation of vital enzymes,[4e,5] (ii) binding to DNA,[6] (iii) interaction with the cell membrane,[7] and (iv) interference with electron transport,[4a] the mechanism of the antimicrobial activity of Ag^+ has not been clarified because of a lack of suitable detection and imaging agents.[8] Ratiometric fluorescent sensors based on pyrene[8d] or BODIPY,[8e] for which a fluorescence shift is observed upon complexation with Ag^+, have been developed; however, they have poor water solubility. Recently, Schmittel et al. reported a luminescent iridium complex capable of detecting Ag^+ in aqueous media; however, this complex also must be used with a mixed solvent of CH_3CN and water.[8g]

In this context, we demonstrate a novel water-soluble fluorescent sensor for Ag^+, RosAg, based on tetramethyl-

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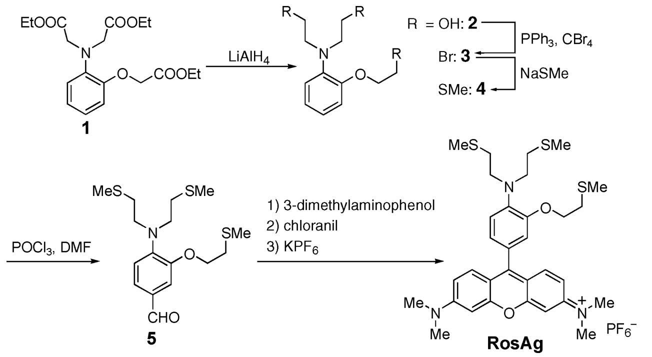
10.1021/ic800442y CCC: $40.75

Scheme 1. Synthesis of RosAg



rosamine, which has been employed as a fluorophore in intracellular Ca^{2+} imaging agents such as Rhod-2.[9] In order to achieve specific binding to Ag^+, we utilized an *o*-aminophenol-*N*,*N*,*O*-triacetic acid derivative consisting of three thioethers instead of the carboxyl group as the chelator and attached it to xanthene at the 9 position. Similar to all Rhod dyes, it is expected that RosAg itself (apo form) will not exhibit fluorescence because of the efficient photoinduced electron transfer (PET) quenching of the xanthene dye by the aniline nitrogen of the chelator moiety.[10]

The synthesis of RosAg is outlined in Scheme 1. Triethyl ester **1**[11] was reduced by $LiAlH_4$ to yield triol **2**, and subsequent bromination using CBr_4 and PPh_3 yielded tribromide **3**. Treatment of tribromide **3** with $NaSCH_3$ produced trithioether **4**, which is the Ag^+ ion binding moiety of the sensor. Successive formylation under Vilsmeier conditions, Friedel−Crafts acylation with 3-(dimethylamino)phenol, chloranil oxidation, and counteranion exchange by KPF_6 yielded RosAg as a dark-purple powder. The structure of the final product was identified by ^1H and ^{13}C NMR, IR spectroscopy, and mass spectroscopy; its purity was confirmed by reversed-phase high-performance liquid chromatography.

Under physiological conditions (50 mM HEPES, pH 7.2, and 0.1 M KNO_3), RosAg exhibited an excitation maximum at 551 nm ($\epsilon = 5.7 \times 10^4\ M^{-1}\ cm^{-1}$) and had a negligible fluorescence quantum yield (<0.005) in the absence of Ag^+. Upon the addition of Ag^+, however, the fluorescence intensity of RosAg showed a ca. 35-fold increase ($\Phi = 0.13$; Figure 1) without a significant change in the excitation and emission maxima ($\lambda_{ex} = 553$ nm; $\lambda_{em} = 574$ nm). The low background fluorescence of RosAg and the large enhancement in the fluorescence of the Ag^+-bound form of this sensor would make it more advantageous than reported on−off-type fluorescent silver sensors, for which the fluorescence intensities show only a 3−4-fold increase.[8g,h] Moreover, the apo and Ag-bound forms of RosAg have stable fluorescence at the biological pH. The fluorescence of RosAg in the absence of Ag^+ did not change above pH 5 and increased as the pH decreased, suggesting that the PET process was inhibited by protonation of the aniline nitrogen of the electron donor.[2a] The fluorescence intensity of RosAg in the presence of Ag^+ was also unchanged in a wide range of pH's (from 2 to 12). We then examined the fluorescence responses of 5 μM RosAg to various biologically relevant metal ions, as shown in Figure 2. Ca^{2+}, Mg^{2+}, Na^+, and K^+, which exist in high concentrations in cells, did not cause an enhancement of the fluorescence even at 5 mM and did not interfere with Ag^+ binding. Surprisingly, a slight enhancement in the fluorescence intensity (ca. 7−8-fold) was observed in the presence of Cu^+ and Cu^{2+}, which are known to be effective fluorescence quenchers.[2a,8a,12] Other transition

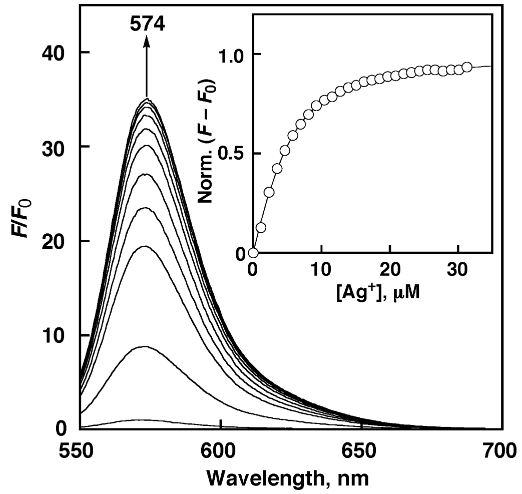


Figure 1. Fluorescence emission spectra ($\lambda_{ex} = 540$ nm) of RosAg (5 μM) as a function of added $AgNO_3$ in 50 mM HEPES (pH 7.20 and 0.1 M KNO_3). The inset shows the fluorescence response at 574 nm, substracting the baseline (F_0) spectrum and normalizing. The obtained data were analyzed by a nonlinear least-squares fitting to determine a dissociation constant of 2.0 μM.

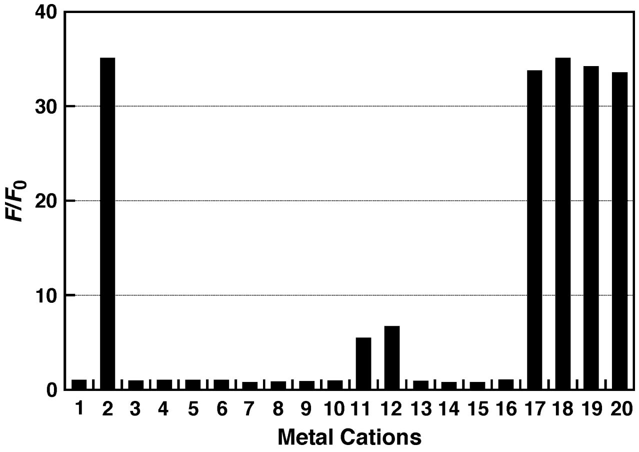


Figure 2. Fluorescence response of RosAg (5 μM) at 574 nm as a function of various added metal cations (5 mM for Na^+, K^+, Ca^{2+}, and Mg^{2+} and 50 μM for all other cations) in 50 mM HEPES (pH 7.20 and 0.1 M KNO_3): 1, no metal; 2, Ag^+; 3, Na^+; 4, K^+; 5, Mg^{2+}; 6, Ca^{2+}; 7, Mn^{2+}; 8, Fe^{2+}; 9, Co^{2+}; 10, Ni^{2+}; 11, Cu^+; 12, Cu^{2+}, 13, Zn^{2+}; 14, Cd^{2+}; 15, Pb^{2+}; 16, Hg^{2+}; 17, $Ag^+ + Na^+$; 18, $Ag^+ + K^+$; 19, $Ag^+ + Mg^{2+}$; 20, $Ag^+ + Ca^{2+}$.

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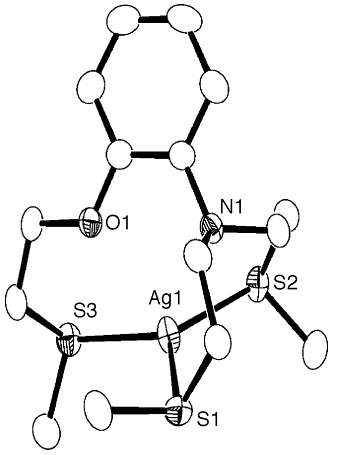

Figure 3. ORTEP diagram of [Ag(**4**)]NO_3. Thermal ellipsoids are shown at 50% probability. H atoms and the nitrate anion are omitted for clarity. Selected bond lengths (Å) and angles (deg): Ag1−S1 = 2.5570(4), Ag1−S2 = 2.5369(3), Ag1−S3 = 2.5421(3), S1−Ag1−S2 = 121.944(12), S1−Ag1−S3 = 115.802(11), S2−Ag1−S3 = 121.424(12).

metals including Fe^{2+}, Mn^{2+}, Pb^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}, and Hg^{2+} had no effect on the fluorescence spectrum. Such photophysical properties in a neutral aqueous solution and the Ag^+ specificity of RosAg suggest that this sensor may be useful in biological applications.

Job's plot monitored by fluorescence intensities indicated the formation of a 1:1 complex. Thus, the dissociation constant ($K_d = 2.0\ \mu$M) for Ag^+ was determined by plotting the fluorescence intensity ($F - F_0$) against [Ag^+] and fitting these data[13] (inset of Figure 1). This K_d value is comparable to the affinities of the reported compounds containing a tetrathia receptor.[8e,i] The Ag^+ affinity and fluorescent properties of RosAg indicate that the aniline nitrogen of the chelator as well as three thioether groups is likely to coordinate to the metal ion in the aqueous solution.

In order to investigate the structural properties of the Ag^+ complex of RosAg, we synthesized the silver complex using a truncated part of the sensor that included its metal binding moiety (trithioether **4**) and determined its crystal structure by X-ray diffraction analysis.[14] As observed in the titration for the Ag^+ complexation to RosAg, [Ag(**4**)]NO_3 was a monomeric silver complex containing a single nitrate anion (Figure 3). The coordination geometry of the Ag atom is almost an ideal trigonal-planar structure (the sum of the angles around Ag is 359.2°) with three coordination sites occupied by S atoms.[15] The metal center lies slightly above the plane formed with the three S atoms ($\rho = 0.1343$ Å). The nitrate anion of [Ag(**4**)]NO_3 was considered to be noninteracting because the shortest Ag−ONO_2 distance is 3.24 Å. Surprisingly, we found that there was no direct coordinative interaction between the Ag and N atom of the ligand [the distance is 2.7254(9) Å]. This result may contradict the observation of the enhancement in fluorescence caused by Ag^+. Therefore, the Ag^+ complexation behavior of **4** in solution was further examined by ^{1}H NMR measurement to confirm whether Ag^+ can approach more closely and interact electrostatically with the N atom of the ligand.[8i,16] The spectrum of [Ag(**4**)]NO_3 in DMSO-d_6 exhibited large downfield shifts of the aromatic protons, particularly for the ortho and para positions toward the amino group, as well as terminal methyl protons adjacent to the S atoms. These shifts are indicative of the strong interactions of Ag−N and Ag−S in the solution. The results obtained are consistent with the increase in the fluorescence intensity upon complexation with Ag^+ because the PET quenching process would be inhibited by the decrease of the electron density of the donor.

In summary, we have developed a new rosamine-based fluorescent sensor (RosAg) for Ag^+. This sensor is soluble in water and can be excited with visible light. It also has pH-independent fluorescence at the biological pH and a large signal-to-noise ratio. To evaluate the suitability of RosAg for biological applications, intracellular imaging of the Ag ion using this sensor is in progress.

Acknowledgment. This work was financially supported in part by Grants-in-Aid for Scientific Research (No. 19750139 to M.T.; No. 19350084 to Y.Y.) from the Japan Society for the Promotion of Science (JSPS) and the Ministry of Education, Culture, Sports, Science, and Technology (MEXT). S.I. thanks the Kyoto University Venture Business Laboratory (KU-VBL) for support.

Supporting Information Available: Experimental details for synthetic procedures, Job's plot, fluorescence pH sensitivity, X-ray diffraction analysis, and ^{1}H NMR spectra of **4** and [Ag(**4**)]NO_3 (PDF) and crystallographic data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) See the Supporting Information.

(14) Crystal data for [Ag(**4**)]NO_3: $C_{15}H_{25}O_4N_2S_3Ag_1$; monoclinic; $P2_1/c$ (No. 14); $a = 9.3704(8)$ Å, $b = 10.1330(9)$ Å, $c = 20.4772(17)$ Å, $\beta = 90.3451(18)°$, $V = 1944.3(3)$ Å^3; $Z = 4$; $T = 203$ K; $\lambda = 0.710\,75$ Å; $\mu = 13.781$ cm^{-1}; $D_{calcd} = 1.713$ g cm^{-3}; R1 = 0.0312 [$I > 2\sigma(I)$]; wR2 = 0.0859; GOF (on F^2) = 1.014. Additional crystallographic details are contained in the Supporting Information.

(15) The trigonal-planar coordination of Ag^+ occupied by S atoms has been rarely reported. For example, see. Mascal, M.; Kerdelhue, J. L.; Blake, A. J.; Cooke, P. A. *Angew. Chem., Int. Ed.* **1999**, *38*, 1968–1971. Fujisawa, K.; Imai, S.; Suzuki, S.; Moro-oka, Y.; Miyashita, Y.; Yamada, Y.; Okamoto, K. *J. Inorg. Biochem.* **2000**, *82*, 229–238.

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