

## Selective Fluorogenic and Chromogenic Probe for Detection of Silver Ions and Silver Nanoparticles in Aqueous Media

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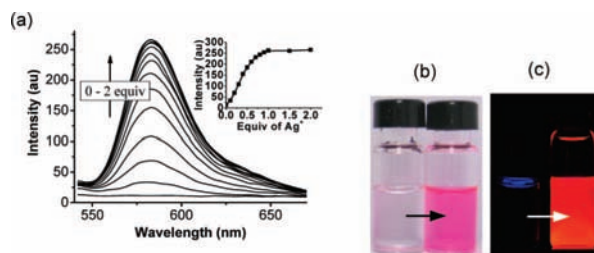
Use of nanomaterials is broadly becoming a part of daily life in the form of biomedicine, biosensors, drug delivery systems, cosmetics, etc.<sup>1</sup> For years, knowledge about silver's ability to kill harmful bacteria<sup>2</sup> makes its nanoparticles popular to create various products.<sup>3</sup> For example, silver nanoparticles (AgNPs) are widely used in consumer products such as detergents and wound dressings, which are finally released into the sewage line and eventually to river streams. However, the positive effects of the silver-related technologies may cause a potentially negative impact on the environment.<sup>4</sup> AgNPs are known to generate highly reactive oxygen species and may destroy the environmentally benign bacteria by inhibiting their growth and disturbing their reproductive ability.<sup>5</sup> Silver ions also bind with various metabolites and inactivate sulfhydryl enzymes.<sup>5</sup>

Atomic absorption and plasma emission spectroscopy as well as anodic stripping voltammetric methods have been used to measure trace levels of silver.<sup>4c,6</sup> Most of these methods are expensive and time-consuming in practice. Therefore, in addition to the sophisticated methods, simpler detection tools such as fluorogenic or chromogenic probes are in demand for the detection of Ag<sup>+</sup> and AgNPs in various situations.

Although considerable efforts have been devoted for the development of fluorescent chemosensors specific for cations,<sup>7</sup> there are only a handful of reports on molecular probes for Ag<sup>+</sup> ions<sup>8</sup> and, to the best of our knowledge, none of them have been applied for the detection of AgNPs. Since colloidal silver can be equilibrated to Ag<sup>+</sup> by oxidation with hydrogen peroxide,<sup>9</sup> a sensing system for Ag<sup>+</sup> may be explored for the detection of AgNPs if the probe survives the oxidation conditions. To this end we have devised a Rhodamine B derivative **1** as the fluorogenic and chromogenic probe for Ag<sup>+</sup>/AgNPs in aqueous media. We reasoned that probe **1** would undergo oxazoline formation upon binding Ag<sup>+</sup> to the iodide,<sup>10</sup> which facilitates the spirolactam ring opening (Scheme 1).<sup>11</sup> Furthermore, the binding-promoted ring-opening process seems to be orthogonal to the oxidation condition of AgNPs, which would eventually enable us to detect AgNPs with probe **1**.

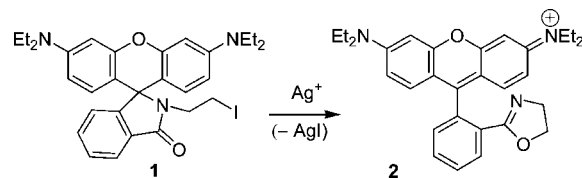
The rhodamine derivative **1**, prepared from Rhodamine B through a two-step procedure ((1) NH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OH, EtOH, 120 °C, 89%; (2a) MsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>; (2b) NaI, acetone, 82%), is stable over 6 months when kept in a refrigerator at -20 °C. Probe **1** forms a colorless and nonfluorescent solution in 20% ethanolic water. Addition of Ag<sup>+</sup> ion to this solution leads to the development of a pink color ( $\lambda_{\text{max}}$ : 558 nm) and a strong orange fluorescence ( $\lambda_{\text{max}}$ : 584 nm), indicating that the Ag<sup>+</sup>-promoted ring opening takes place readily. When 1.0 equiv of probe **1** was used, the fluorescence gradually increased and saturated within 3 h.

For a faster measurement, more than 1 equiv of the probe can be used because the fluorescence of probe **1** itself is negligible compared to that of oxazoline **2**. For example, the fluorescence



**Figure 1.** (a) Fluorescence response of **1** (10  $\mu\text{M}$ ) after 10 min upon addition of 0–2 equiv of Ag<sup>+</sup> in 20% ethanolic water at 25 °C (excitation at 530 nm). Inset: a fluorescence intensity plot depending on the equiv of Ag<sup>+</sup>. (b) Color change and (c) fluorescence change of **1** (50  $\mu\text{M}$ ) upon addition of 1.0 equiv of Ag<sup>+</sup> in 20% ethanolic water, after 10 min.

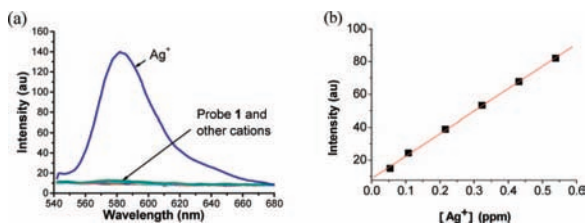
### Scheme 1. Ag<sup>+</sup> Promoted Spirolactam Ring Opening of Probe **1**



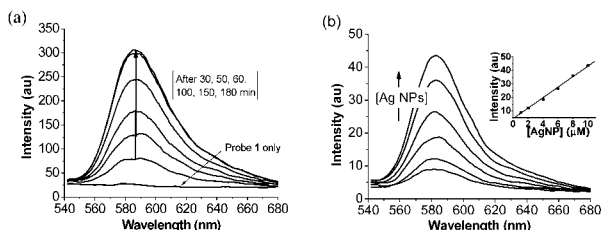
saturation was attained within 10 min when 10 equiv of probe **1** were used. Probe **1** and Ag<sup>+</sup> react in a 1:1 stoichiometry (Figure 1a inset). The formation of oxazoline **2** was confirmed by synthesizing it separately from an equimolar mixture of **1** and AgNO<sub>3</sub> in methanol. A high quantum yield ( $\Phi_f = 0.71$ ) and a large molar absorptivity ( $\log \epsilon = 4.33$ ) of oxazoline **2** confirm that the fluorescence enhancement is due to its formation. As probe **1** reacts with Ag<sup>+</sup>, both color change to pink and “turn-on” type fluorescence change to orange (Figure 1b and 1c) are observed. Counteranions of silver salts have a negligible effect on the sensing process, as AgOAc and AgClO<sub>4</sub> gave similar results.

The fluorescence response of probe **1** toward various other metal ions (Ca<sup>2+</sup>, Ba<sup>2+</sup>, Mg<sup>2+</sup>, Cr<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>3+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Pd<sup>2+</sup>, Cd<sup>2+</sup>, Hg<sup>+</sup>, and Hg<sup>2+</sup>) was also examined; however, metal ions other than Ag<sup>+</sup> showed little spectral or color changes (Figure 2a). A similar response was observed when Ag<sup>+</sup> ion was added to the probe solution in the presence of all other metal ions. Such an excellent selectivity of probe **1** toward Ag<sup>+</sup> among other metal ions may be explained by the specific molecular interaction between Ag<sup>+</sup> ion and the iodide.

The fluorescence response of probe **1** toward Ag<sup>+</sup> was linear when measured in the range 0.1–5.0  $\mu\text{M}$  (0.011–0.54 ppm) of Ag<sup>+</sup> (a part of the graph is shown in Figure 2b), and a signal-to-noise ratio of 3 was observed up to 0.13  $\mu\text{M}$  of Ag<sup>+</sup>. From these data, we have estimated the detection limit of probe **1** to be 14 ppb.<sup>12</sup>



**Figure 2.** (a) Fluorescence spectral change of **1** (10  $\mu\text{M}$ ) when treated with 1.0 equiv of each metal ion, taken after 1 h of each addition. (b) A fluorescence intensity profile of **1** upon addition of  $\text{Ag}^+$  (0.050–0.54 ppm) (the intensity was taken at the peak height at 584 nm). Both data were obtained in 20% ethanolic water at room temperature.



**Figure 3.** (a) Changes in fluorescence spectra of **1** (20  $\mu\text{M}$ ) upon addition of AgNPs (10  $\mu\text{M}$ ) in the presence of 1.0 mM  $\text{H}_2\text{O}_2$  and 1.0  $\mu\text{M}$   $\text{H}_3\text{PO}_4$ . (b) Fluorescence intensity changes of **1** after 1 h upon addition of AgNPs (1.0, 2.0, 4.0, 6.0, 8.0, and 10  $\mu\text{M}$  each) in the presence of the acidic  $\text{H}_2\text{O}_2$  solution. Inset: changes in the fluorescence intensity profile of **1** at 584 nm.

The cytotoxicity of probe **1** was examined toward HeLa cells by an MTS assay, showing little cytotoxicity up to 100  $\mu\text{M}$  level for 24 h.

For the detection of AgNPs using probe **1**, we have utilized the oxidation reaction of AgNPs by  $\text{H}_2\text{O}_2$  under acidic conditions,<sup>9</sup> which leads to the equilibration of AgNPs to  $\text{Ag}^+$  ions. Thus, to a solution of 1.0 mM  $\text{H}_2\text{O}_2$  and 1.0  $\mu\text{M}$   $\text{H}_3\text{PO}_4$  in 20% ethanolic water were added a 10  $\mu\text{M}$  solution of AgNPs ( $8 \pm 1$  nm) and probe **1**, and then the fluorescence of the solution was measured at given time intervals. The reaction time for the formation of oxazoline **2** can be also shortened by increasing the amount of the probe used. Figure 3a shows the fluorescence change for the silver nanoparticle solution when 2.0 equiv of probe **1** were used. The fluorescence increased linearly depending on the concentration of AgNPs (Figure 3b), demonstrating the usefulness of probe **1** for the quantification of AgNPs. Without AgNPs, the probe remains unaffected under otherwise the same conditions.

The nanoparticle sensing protocol is applied for the quantification of AgNPs in consumer products. A hand sanitizer gel and fabric softener are chosen among various items, both of which contain AgNPs but the amounts of which are not specified.<sup>13</sup> Thus, a measured amount of the gel was treated with aqueous  $\text{H}_2\text{O}_2$  and  $\text{H}_3\text{PO}_4$  followed by probe **1**, and the resulting solution was kept closed for 1 h at 25  $^\circ\text{C}$ , resulting in the complete formation of oxazoline **2**. Then, oxazoline **2** in the solution was extracted with ethyl acetate and subjected to the fluorescence measurement. On the basis of a calibration curve obtained separately from known concentrations of AgNPs solutions, we were able to determine the concentration of AgNPs to be 1.9 ppm. Similarly, the concentration of AgNPs in the fabric softener was determined to be 0.6 ppm. Separate inductively coupled plasma atomic emission spectroscopy analyses for each sample, which required complex and tedious sample preparation procedures, gave the same values within

experimental errors. The results clearly demonstrate that probe **1** provides a simple method for quantification of AgNPs in consumer products.

In summary, we have disclosed a rhodamine-based fluorogenic and chromogenic probe for  $\text{Ag}^+$  ions that is also applicable for the detection of AgNPs for the first time. The sensing mechanism is based on irreversible tandem ring-opening and -forming processes promoted by  $\text{Ag}^+$ -coordination to the iodide of the probe, which is accompanied by both color and turn-on type fluorescence changes. The probe shows remarkably high selectivity over other metal ions and detects silver ions up to 14 ppb. Furthermore, the sensing protocol can be applied for the quantification of AgNPs in consumer products.

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**Supporting Information Available:** Synthesis and characterization of compounds **1** and **2**, and the experimental procedures for the determination of detection limit and concentrations of AgNPs in the commercial samples. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (12) The MCL (maximum contamination level) limit for silver is 0.1 ppm (100  $\mu\text{g/L}$ ) according to USEPA 2001.
- (13) Most of the consumer products searched by us contain AgNPs whose size is in the range 1–10 nm.

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