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A Gold(III) Ion-Selective Fluorescent Probe and Its Application to Bioimagings

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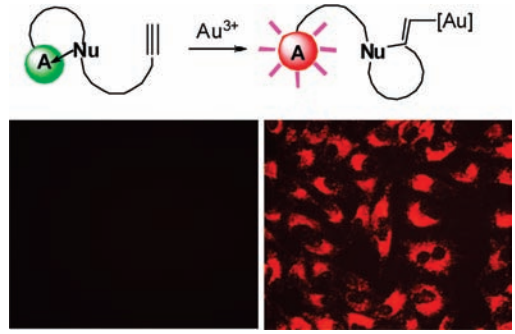
ABSTRACT



A highly selective and sensitive fluorescent chemosensor for Au^{3+} has been reported. The system utilizes an irreversible Au^{3+}-promoted cyclization reaction of a rhodamine amide tethered with an alkyne. The probe can sense Au^{3+} ions selectively over other biologically relevant metal ions, and ~50 nM of Au^{3+} could be readily detected in aqueous media. Fluorescent imaging of Au^{3+} in living cells is also successfully demonstrated.

Recently, gold and gold salts have been widely applied in the fields of nanomaterials and catalysis. Because of high biocompatibility, functionalized gold nanoparticles[1] have been extensively used as drug and gene delivery systems,[2] biosensors,[3] and bioimaging materials.[4] Although metallic gold is stable and highly biocompatible, its ionic forms are very reactive and potentially toxic to humans.[5] Au^{+} and Au^{3+} are the most stable forms out of several possible ionic states of gold. Metallic gold can be chemically oxidized to ionic states by alkaline solutions of cyanide or by dissolving in aqua regia (a mixture of nitric and hydrochloric acids).[6] Not only bioreduction of gold ions to metallic gold (especially gold nanoparticles)[7] but also oxidation of Au^{+} to Au^{3+} in biological systems in the presence of strong oxidants, such as H_2O_2 or ^{-}OCl,[8] are known in the literature. Although there is no sufficient evidence that Au^{0} could be oxidized to Au^{+} or Au^{3+} in biological environments, the long-term in vivo

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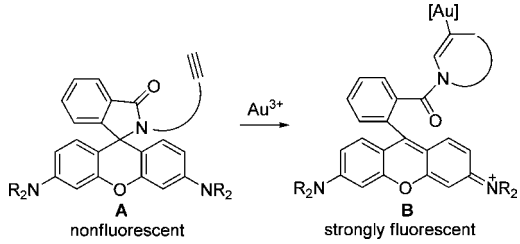
stability and cytotoxicity of gold nanoparticles are under critical debate. Since Au^{+}- and Au^{3+}-based drugs[9] and gold nanoparticle-based materials are currently in use in various fields, the toxic effects of gold species should be properly addressed. In particular, Au^{3+} ions are known to be highly toxic in the biological systems because they bind strongly to DNA and nervous systems and can even cause DNA cleavage.[5] Thus, it is highly desirable to develop detection systems for real-time monitoring of gold ions in environmental and biological samples.

Because fluorescence-based chemosensors are simple and highly sensitive, they have been widely applied in the detection of many biologically relevant metal ions in recent years. In particular, toxic metal ions, such as Hg^{2+},[10] Pb^{2+},[11] Ag^{+},[12] and Cd^{2+},[13] have been successfully monitored by using fluorescent probe technique. Despite considerable recent interest and advances in gold-related research, there have been no reports on fluorescent probes for the detection of gold ions.

Due to the strong alkynophilicy of gold ions, the gold-catalyzed organic transformations have boomed in the past few years.[14] The highly electrophilic gold−alkyne complexes are labile toward a variety of nucleophiles. Thus, the intramolecular cyclization of the alkynes activated by gold ions could be utilized in the design of reaction-based irreversible fluorescent chemosensors.[15] We envisioned that a rhodamine amide tethered with an alkyne could be used to detect gold ions (Scheme 1), where activation of the alkyne

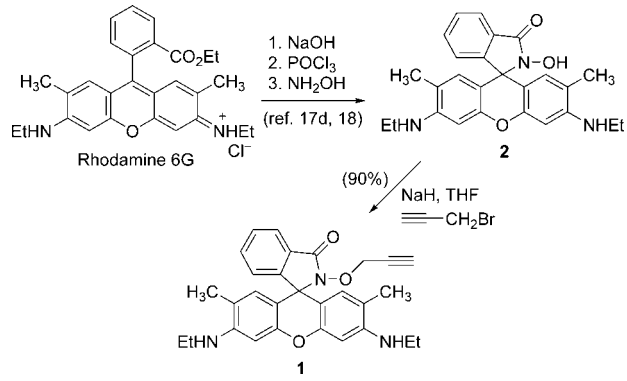
Scheme 1. Schematic Representation of a Reaction-Based Fluorescent Sensing of Gold Ions



with gold ions would convert the nonfluorescent spirocyclic form (**A**) into the fluorescent ring-opened one (**B**).[16,17] Herein, we present a highly selective fluorogenic and chromogenic probe for Au^{3+} ions based on the irreversible chemical reaction between alkynes and gold ions.

We screened several rhodamine amide derivatives tethered with an alkyne or allene functionality and decided to use the alkyne-tethered hydraxamate **1** as the gold ion probe for our studies. Probe **1** is synthesized from the known rhodamine hydroxamic acid **2**[17d,18] which is prepared from rhodamine 6G as shown in Scheme 2. Probe **1** shows neither color nor fluorescence in PBS buffer (1% MeOH) solution indicating that it exists in the spirocyclic form predominantly as expected. On treatment with 2 equiv of Au^{3+} ions, probe **1** (20 μM) exerts a new UV absorption band at 524 nm in PBS buffer (1% MeOH) solution. In addition, the solution

Scheme 2. Synthesis of Rhodamine Probe **1**



changes from colorless to a pink-red color. Probe **1** functions well to sense Au^{3+} in the pH 7−10 range (see the Supporting Information).

A fluorescence titration of Au^{3+} was conducted using a 20 μM solution of **1** in PBS buffer (1% MeOH) at pH 7.4. After each addition of Au^{3+} solution to **1**, the solution was incubated for 25 min,[19] and then fluorescence intensity is measured. About 2 equiv of Au^{3+} is required for the

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(19) It took 60−80 min for the completion of the fluorescence intensity changes with 2 equiv of Au^{3+} as shown in the Supporting Information. However, in the titration experiment, where 0.2 equiv of Au^{3+} is treated each time, the fluorescence changes were almost complete in 25 min.

saturation of the fluorescence intensity under the titration conditions (Figure 1a). The observed fluorescence intensity

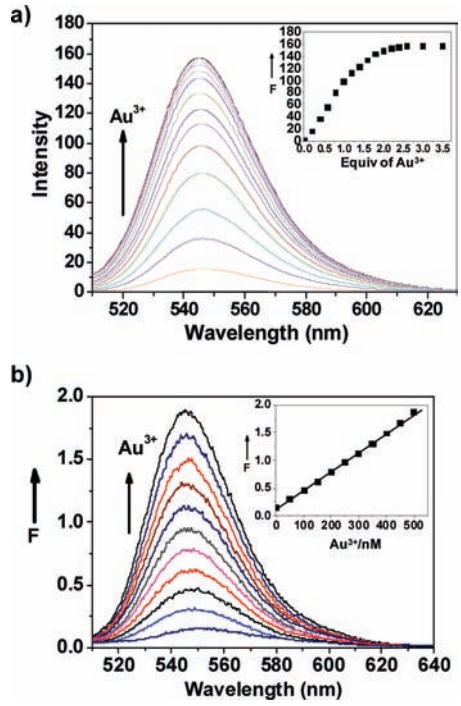

Figure 1. (a) Fluorescence response of **1** (20 μM) upon addition of Au^{3+} in PBS buffer (1% MeOH) at pH 7.4 (excitation at 500 nm, 25 °C). Inset: plot of fluorescence intensity depending on the number of equivalents of Au^{3+}. (b) Fluorescence response of **1** (1 μM) upon addition of Au^{3+} (by 50 nM) in PBS buffer (0.1% MeOH) at pH 7.4 (25 °C, excitation at 500 nm). Inset: plot of fluorescence intensity at 545 nm depending on the concentration of Au^{3+}. Each spectrum is acquired after 25 min of each Au^{3+} addition.

(at 545 nm) is nearly linearly proportional to the concentration of Au^{3+}. It seems that the reaction between probe **1** and Au^{3+} is not catalytic because the catalytic cycle does not operate under the reaction conditions (low temperature and aqueous media).

We then attempted to isolate the product of the reaction between probe **1** and Au^{3+} ions. Although probe **1** is completely consumed upon treatment with $AuCl_3$ (or $HAuCl_4$) in CH_2Cl_2, we could not isolate the expected product(s).[20] According to the ^1H NMR titration experiment, however, the acetylene proton peak (at 2.32 ppm) clearly disappears upon addition of 1 equiv of Au^{3+} ions (see the Supporting Information). To see whether the process is reversible, excess amounts of CN^- ions were added into the titration solution to liberate gold cyanide complexes. However, the fluorescence intensity of the solution did not change at all (see the Supporting Information). Therefore, it seems that the Au^{3+} ion-mediated cyclization process is not reversible. Once the fluorescent cyclization intermediate is formed, the total fluorescence intensity maintains whether the intermediate undergoes further reactions or not. The fluorescence titration experiment of solution **1** (1 μM) with Au^{3+} demonstrates that the detection of Au^{3+} is possible at the 50 nM level (Figure 1b). Under these conditions, the fluorescence intensity of the solution is linearly proportional to the concentration of Au^{3+}.

Next, the fluorescence responses of **1** (20 μM) to other biologically relevant metal ions in PBS buffer (1% MeOH) at pH 7.4 were examined. Upon addition of 2 equiv of metal ions (Hg^{2+}, Zn^{2+}, Pb^{2+}, Ca^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Mg^{2+}, Cd^{2+}, Cu^{2+}, Fe^{3+}, Cr^{3+}, Al^{3+}, Au^{+}, Ag^{+}, Na^{+}, and Li^{+}), no significant fluorescence intensity changes were observed (Figure 2a). Only Au^{3+} leads to a dramatic enhancement in

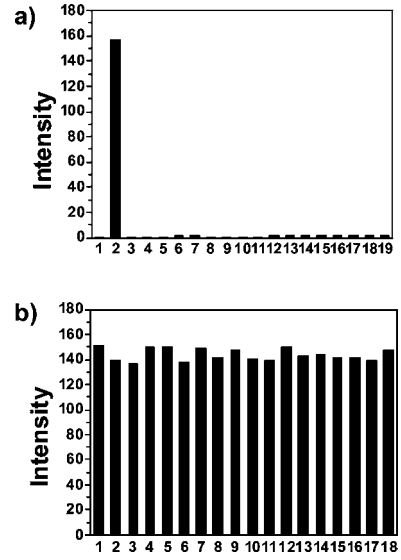

Figure 2. Fluorescence responses of **1** in the presence of metal ions. (a) Fluorescence spectra (excitation at 500 nm; emission at 545 nm) of **1** (20 μM) in PBS buffer (1% MeOH) at pH 7.4 in the presence of 2 equiv of metal ions: 1, none; 2, Au^{3+}; 3, Hg^{2+}; 4, Zn^{2+}; 5, Pb^{2+}; 6, Ca^{2+}; 7, Co^{2+}; 8, Fe^{2+}; 9, Mn^{2+}; 10, Mg^{2+}; 11, Cu^{2+}; 12, Cd^{2+}; 13, Fe^{3+}; 14, Al^{3+}; 15, Cr^{3+}; 16, Au^{+}; 17, Ag^{+}; 18, Na^{+}; 19, Li^{+}. (b) Fluorescence intensities of **1** (20 μM) in PBS buffer (1% MeOH) at 545 nm (excitation at 500 nm, 25 °C) in the presence of Au^{3+} (2.0 equiv) and 4.0 equiv the other metal ions: 1, none; 2, Hg^{2+}; 3, Zn^{2+}; 4, Au^{+}; 5, Pb^{2+}; 6, Ca^{2+}; 7, Fe^{3+}; 8, Cr^{3+}; 9, Co^{2+}; 10, Mg^{2+}; 11, Mn^{2+}; 12, Cd^{2+}; 13, Fe^{2+}; 14, Ag^{+}; 15, Li^{+}; 16, Al^{3+}; 17, Cu^{2+}; 18, Na^{+}.

fluorescence intensity, and the fluorescence intensity changes caused by the addition of Au^{3+} are not influenced by the presence of other metal ions as shown in Figure 2b. Interestingly, the observed selectivities for Au^{3+} over other electrophilic metal ions, such as Au^{+}, Hg^{2+}, and Ag^{+}, is remarkably high in aqueous buffer solutions. Because the fluorescent probe **1** is highly selective and sensitive toward Au^{3+}, it could be ideal for the biological imaging applications.

Then, we studied bioimaging applications of **1** for monitoring of Au^{3+} ions in biological systems. HeLa cells were

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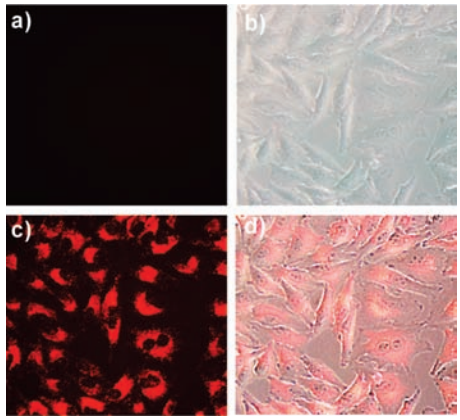

Figure 3. Images of HeLa cells: (a) fluorescence image of HeLa cells treated with **1** (20 μM) in the absence of Au^{3+} (control); (b) microscopic image of HeLa cells treated with Au^{3+} (10 μM) and **1** (20 μM); (c) fluorescence image of HeLa cells treated with Au^{3+} (10 μM) and **1** (20 μM); (d) merge image of frames b and c.

incubated with and without Au^{3+} (5−20 μM) for 25 min at 37 °C and washed with PBS buffer (pH 7.4) to remove the remaining Au^{3+}. Then the treated cells were incubated with **1** (20 μM) in the culture medium for 30 min at 37 °C. After incubation under the conditions, the cells were washed with PBS buffer and imaged using a fluorescence microscope. The cells not treated with Au^{3+} show no fluorescence (Figure 3a), but the cells exposed to both Au^{3+} and **1** display strong red fluorescence (Figure 3c). The fluorescence intensities increase as the concentration of Au^{3+} increase (see the Supporting Information).

In conclusion, we have described a highly selective and sensitive fluorescent chemosensor for the detection of Au^{3+} in aqueous media for the first time. The system utilizes an irreversible Au^{3+}-promoted cyclization reaction of a rhodamine amide tethered with an alkyne. The probe can sense Au^{3+} ions selectively over other biologically relevant metal ions, and ∼50 nM of Au^{3+} could be readily detected in aqueous media. Fluorescence imaging of Au^{3+} in living cells is also successfully demonstrated. We anticipate that the present fluorescent probe could serve as a new tool in gold ion-related chemical and biological studies.

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Supporting Information Available: Experimental procedures for the synthesis, spectral data, and copies of ^{1}H NMR and ^{13}C NMR of **1**; data for UV−vis and fluorescence titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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