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A fluorescent probe for copper and hypochlorite based on rhodamine hydrazide framework

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ABSTRACT

A rhodamine derivative (**1**) was synthesized as a fluorescence turn-on probe for copper (Cu^{2+}) and hypochlorite (ClO^-). The probe gave a fluorescence turn-on change at 587 nm with a color change from colorless to pink in the presence of Cu^{2+} or ClO^- ions in aqueous solution. It was found that the Cu^{2+} ion bound to **1** in a 1:1 stoichiometry and induced a spirolactam ring opening of the rhodamine moiety leading to a fluorescence turn-on, confirmed by Job's plot, ESI-Mass, and ^1H NMR analyses. In the presence of ClO^- ion, probe **1** underwent a hypochlorite-mediated oxidation and hydrolysis to produce a ring-opened rhodamine B with a fluorescence enhancement. However, these changes were not monitored in case of other metal ions, anions, and reactive redox species. In addition, probe **1** can readily react with the ClO^- to provide a distinct fluorescence enhancement along with a pink color even in the presence of various competitive species.

Introduction

Copper is one of the most abundant heavy metals in biological and environmental systems. Especially, copper is an essential metal in human body, it serves as an essential co-factor for several redox-related enzymes including superoxide dismutase, ferroxidases, peroxidase, and dopamine β-monooxygenase and plays a crucial role in various biological redox processes.[1] However, excessive copper is associated with cellular damage, causing a variety of diseases including Alzheimer's[2] and Wilson's diseases.[3] Because of the toxicity of copper, the environmental protection agency (EPA) limits copper concentrations to 1.3 mg/L in drinking water.[4] On the other hand, hypochlorite ion (ClO^-) is one of reactive oxygen species (ROS) and acts as an antimicrobial agent in the immune system.[5] However, excessive generation of ClO^- is related with tissue damage, many immunity-associated diseases, including lung injury atherosclerosis,[6] osteoarthritis,[7] and various cancers.[8] The EPA also regulate hypochlorite analogues such as chlorate (ClO_3^-), chlorite (ClO_2^-), chlorine (Cl_2), chlorine dioxide (ClO_2) in drinking water to 0.3, 1.0, 4.0, 0.8 mg/L respectively.[9] Therefore, an effective detection of Cu^{2+} and ClO^- ions from biospecimens and drinking water is crucially essential for human being.

So far, some conventional analytical methods such as atomic adsorption spectrometry,[10] X-ray fluorescence spectrometry,[11] and laser-induced breakdown spectroscopy[12] were utilized to detect copper ions in biological and environmental samples. However, there are several limitations including high cost, operational expertise, and lack of portability. Currently, fluorescent small molecule-based probes are developed for the sensing of Cu^{2+} and ClO^- ions.[13–15] However, only a few fluorescent sensors are able to detect both Cu^{2+} and ClO^- ions. In addition, we think that a fluorescent probe that can detect both Cu^{2+} and ClO^- ions based on a different sensing mechanism would be interesting and it could be applicable to a variety of sensing and imaging fields.

Herein, a rhodamine hydrazide derivative (**1**) was developed as a fluorescence turn-on probe for Cu^{2+} and ClO^- ions. This probe gave a fluorescence turn-on signal at 587 nm with a color change from colorless to pink in the presence of Cu^{2+} or ClO^- ions. As depicted in Scheme 1, the operating principles of **1** for Cu^{2+} and ClO^- are based on a Cu^{2+} binding-induced ring opening of the rhodamine and a hypochlorite-induced hydrolysis producing a ring-opened rhodamine B, respectively. In addition, probe **1** can readily react with ClO^- ions to provide a fluorescence increase and pink color even in the coexistence of various interferants such as metal ions including Cu^{2+}, anions, and reactive redox species. The sensing ability and mechanism of **1** towards Cu^{2+} and ClO^- were identified by absorption and fluorescence spectroscopy, ESI-Mass and ^1H NMR analyses.

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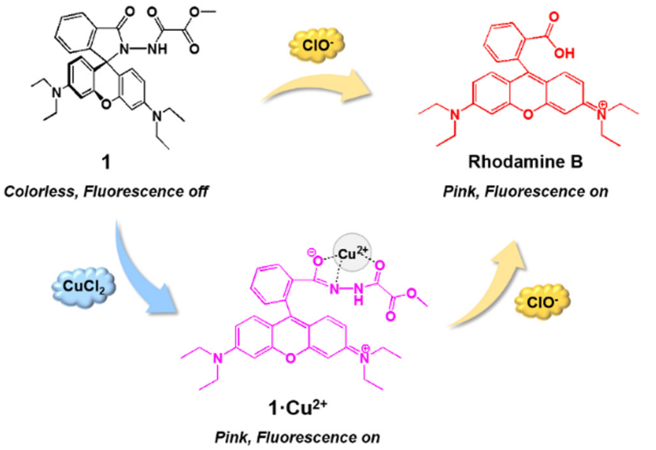

Scheme 1. Schematic illustration of Cu^{2+} and ClO^- sensing mechanism for probe **1**.

Results and discussion

Reagents and materials

All reagents, such as chloride salts of metal ions, $[Cu(CH_3CN)_4]PF_6$, $Cu(ClO_4)_2$, tetrabutylammonium (TBA) salts of anions, glutathione (GSH), cysteine (Cys), homocysteine (Hcy), sodium hydrosulfide for SH_2 generation, reagents for reactive oxygen species generation, and other chemicals for synthesis and analysis were purchased from Aldrich, TCI, Alfa and used as received. All solvents were HPLC reagent grade, and distilled water was used in the analytical experiments. NMR was recorded at Varian 400 MHz and Bruker 500 MHz instrument and all chemical shifts are reported in ppm value using TMS as an internal reference. HR-ESI-MS data were obtained using liquid chromatography mass spectrometer (LC/MS) at the Korea Basic Science Institute.

UV/Vis absorption and fluorescence spectroscopy

Stock solutions of metal chloride and perchlorate salts were prepared in deionized water. Stock solutions of probe, TBA salts of anions, $[Cu(CH_3CN)_4]PF_6$ for Cu^+ ion were prepared in CH_3CN. Stock solutions of thiols were prepared in phosphate buffered saline (PBS) (10 mM, pH 7.4). Stock solutions of reactive oxygen species were prepared by using literature procedures.[16] All spectra were recorded in HEPES (10 mM, pH 7.4):CH_3CN (40:60, v/v). UV/Vis absorption and fluorescence spectra were recorded using UV-2600 (Shimadzu), and RF-6000 (Shimadzu) spectrophotometers, respectively. Excitation was carried out at 550 nm with all excitation and emission slit widths at 5 nm.

X-ray crystallography

Single crystals of **1** suitable for X-ray analysis were obtained by slow diffusion of Et_2O into a CH_3OH solution of **1**. Single crystals of **1** were picked from solutions by a nylon loop (Hampton Research Co.) on a handmade copper plate and mounted on a goniometer head in a N_2 cryostream. Data collections were carried out at 100 (2) K on a Bruker SMART AXS diffractometer equipped with graphite-monochromated Mo $K\alpha$ radiation ($\lambda = 0.71073$ Å). The CCD data were integrated and scaled using the Bruker-SAINT software package. The structure was solved with the program SHELXS-97 (Sheldrick, 2008) and were refined on F^2 with SHELXL-97 (Sheldrick, 2008).[17] All details of the refinement are provided in the CIF files. The CCDC-1543479 contains the Supplementary crystallographic data for **1** (Tables S1 and S2). These data can be obtained free of charge via www.ccdc.cam.ac.uk/ data_request/cif or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk.

Synthetic procedures

Synthesis of **2**: Compound **2** was prepared by according to a literature procedure.[18]

Synthesis of **1**: Compound **2** (0.5 g, 1.1 mmol) and methyl oxalyl chloride (0.152 ml, 1.65 mmol) were dissolved in dichloromethane (DCM) (20 ml). Triethylamine (TEA) (0.3 ml, 2.2 mmol) was added to a mixture and then refluxed for 5 h under nitrogen atmosphere. The reaction solvent was evaporated, and the crude product dissolved in DCM and washed with water twice. The organic layer was collected, dried with anhydrous sodium sulfate, and filtered. After evaporation of the organic solvent, the crude product was purified by a silica column chromatography using ethyl acetate/hexanes (2:1, v/v) as the eluent, and yielded **1** as a brown solid (0.40 g, 67%). ESI-MS m/z $[M+H]^+$ calc. 543.25, obs. 543.10. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.20 (t, $J = 5.1$ Hz, 12H); 3.43–3.19 (m, 8H); 3.79 (s, 3H); 6.42–6.18 (m, 4H); 6.68 (d, $J = 8.8$ Hz, 2H); 7.09 (d, $J = 7.0$ Hz, 1H); 7.56–7.39 (m, 2H); 7.95 (m, $J = 7.1$ Hz, 2H); 8.21 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 164.5, 159.7, 153.9, 153.5, 152.0, 149.0, 133.4, 129.0, 128.3, 128.1, 124.0, 123.5, 108.1, 103.5, 97.6, 77.4, 77.1, 76.8, 66.2, 53.5, 44.3, 12.6.

Probe **1** was synthesized as depicted in Scheme 2a. Precursor **2** was prepared by adopting a literature reported previously.[18] Condensation of **2** and methyl oxalyl chloride in the presence of TEA as a base in DCM yielded **1** in 67% yield. The chemical structures of synthetic materials were firmly confirmed by ^1H, ^{13}C NMR, and ESI-MS analyses (Figs. S6–S8). In addition, single crystals of **1** were analyzed by X-ray crystallography and revealed in Scheme 2b, indicating that the **1** exists in a spirolactam ring-closed form.

Optical properties of 1

To check the sensing ability of **1**, absorption and fluorescence changes of **1** were monitored upon addition of various analytes such as metal ions, anions, and reactive redox species. In Fig. 1, probe **1** was tested towards various metal ions including Na^+, K^+, Mg^{2+}, Ca^{2+}, Ni^{2+}, Zn^{2+}, Cu^+, Cu^{2+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Hg^{2+}, Pb^{2+}, Fe^{2+}, and Fe^{3+} in HEPES buffer (10 mM, pH 7.4)/CH_3CN (40:60, v/v). Initially, a solution of **1** exhibited no absorption and emission bands in a visible region (Fig. 1a and b). However, when Cu^{2+} ions were added into a solution of **1**, absorption band at 550 nm and emission band at 587 nm clearly appeared. The color change was also observed from colorless to pink. In contrast, these changes were not seen in case of other metal ions. Moreover, fluorescence response of **1** to Cu^{2+} ions was investigated as a function of time. In the presence of Cu^{2+} ions, the fluorescence intensity at 587 nm of **1** gradually increased until 95 min and reached a plateau (Fig. S1). Upon increasing concentrations of Cu^{2+} ion, the **1** displayed a gradual increase in the fluorescence intensity and it was saturated at 800 equiv. of Cu^{2+} ions (Fig. 1c). In addition, there was a good linear correlation between the fluorescence intensity (FI) at 587 nm and the $[Cu^{2+}]$ ranging from 0 to 400 equiv. ($R^2 = 0.99347$) (Fig. 1d). The detection limit of **1** for Cu^{2+} was also evaluated to be 0.95 μM, which is below the Cu^{2+} level in the drinking water limited by EPA.[4] These results implied that the **1** can be used as a selective fluorescent probe for Cu^{2+} ions in aqueous solution.

As displayed in Fig. 2, absorption and fluorescence changes of **1** were investigated upon addition of anions (e.g., ClO_4^-, OAc^-, CN^-, HSO_4^-, HPO_4^-, OH^-, F^-, Cl^-, I^-) and redox species such as ROS