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A turn-on fluorescent chemosensor for Cu^{2+} in aqueous media and its application to bioimaging

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ABSTRACT

A rhodamine-based fluorogenic probe bearing the quinaldine unit (**L1**) was developed as a turn-on fluorescent chemosensor for Cu^{2+}. Upon binding with Cu^{2+}, comparable amplifications of absorption and fluorescence signals were observed, which suggest that chemosener **L1** effectively avoided the fluorescence quenching caused by the paramagnetic nature of Cu^{2+}. Importantly, **L1** can selectively respond to Cu^{2+} over other commonly coexistent metal ions (such as K, Ca^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Zn^{2+}, Cr^{3+}) in aqueous media with a rapid response time (<2 min). In addition, biological imaging studies using living cells to monitor Cu^{2+} is successfully demonstrated.

1. Introduction

The production of fluorescent devices for the sensing and reporting of chemical events is currently of significant importance for both chemistry and biology [1]. The growing interest in this field can be rationalized by considering the ever-increasing air and water pollution and the consequent need to monitor the polluting species, such as Hg^{2+}, Pb^{2+}, Cu^{2+}, etc [2–4]. More specifically, sensors directed toward the detection and measurement divalent copper has enjoyed particular attention. Copper is the third most abundant essential trace element in the human body and is commonly found as Cu^{2+} in natural water [5]. However, Cu^{2+} can be toxic to biological systems when levels of Cu^{2+} ions exceed cellular needs, and it is also capable of displacing other metal ions which act as cofactor in enzyme-catalyzed reactions [6]. For these reasons, numbers of fluorescent probes for copper ion have been reported in the past few years. For most the reported Cu^{2+} fluorescent sensors, the binding of the Cu^{2+} ion causes a fluorescence quenching due to its paramagnetic nature [7], although a few examples of "off-on" type sensors available in aqueous systems have also been reported.

Rhodamine as an excellent fluorochrome has attracted considerable interest to prepare various spectroscopic off-on-type probes in recent years by virtue of its long absorption and emission wavelengths elongated to visible region, high fluorescence quantum yield, and large absorption coefficient [8]. The on/off fluorescence switching of these probes is based on structure change of the rhodamine moiety between spirocyclic and openring forms. Without cations, these chemosensors exist in a spirocyclic form, which is colorless and nonfluorescent. In constrast,addition of metal cation leads to a spirocycle opening via coordination resulting in an appearance of pink color and orange fluorescence [9].

2. Experiment procedure

^1H and ^{13}C NMR spectra were taken on a Varian mercury-400 spectrometer with TMS as an internal standard and $CDCl_3$ as solvent. Absorption spectra were determined on a Varian UV-Cary100 spectrophotometer. Fluorescence spectra measurements were performed on a Hitachi F-4500 spectrofluorimeter. All pH measurements were made with a pH-10C digital pH meter. HRMS

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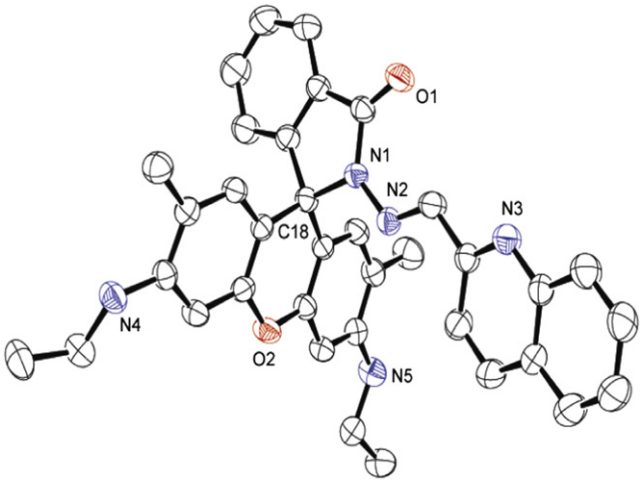

Fig. 1. ORTEP diagram of the compound **L1**. (50% probability level for the thermal ellipsoids).

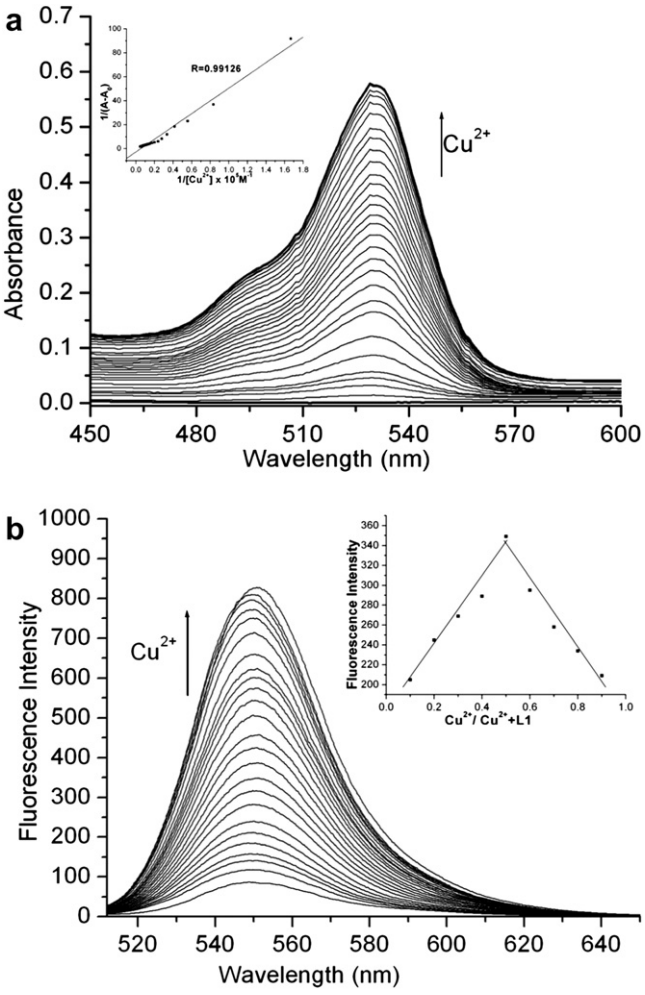

Fig. 2. a) Absorption spectra of **L1** (2×10^{-5} mol L^{-1}) in water/CH_3CN (1:1, v/v) in the presence of different amounts of Cu^{2+} (0–10 equiv). Inset: absorbance at 531 nm as a function of Cu^{2+} concentration b) Fluorescence spectra of **L1** (2×10^{-5} mol L^{-1}) in water/CH_3CN (1:1, v/v) upon addition of Cu^{2+} (0–2.5 equiv). Inset: Job's plots according to the method for continuous variations, indicating the 1:1 stoichiometry for **L1**–Cu^{2+} (the total concentration of **L1** and Cu^{2+} is 2.5×10^{-5} mol L^{-1}). ($\lambda_{ex} = 500$ nm).

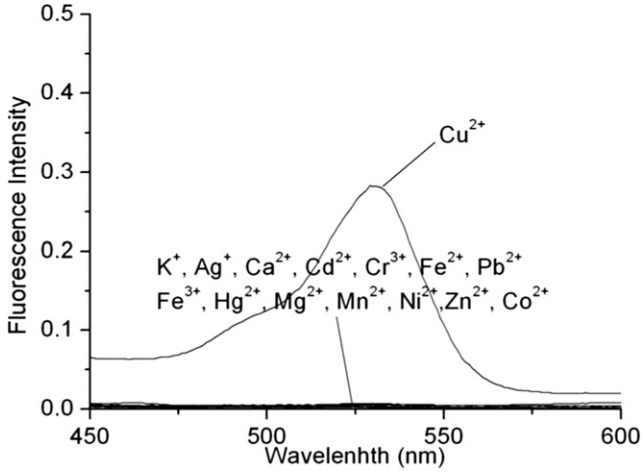

Fig. 3. Changes in the absorption spectra of **L1** (2×10^{-5} mol L^{-1}) in the presence of different metal ions (1×10^{-4} mol L^{-1}) in water/CH_3CN (1:1 v/v).

were determined on a Bruker Daltonics APEXII 47e FT-ICR spectrometer.

All the materials for synthesis were purchased from commercial suppliers and used without further purification. Methanol for spectra detection was HPLC reagent without fluorescent impurity.

Stock solutions of the metal ions (2.5×10^{-3} mol L^{-1}) were prepared in deionized water. A stock solution of **L1** (1×10^{-3} mol L^{-1}) was prepared in DMF: CH_3CN (1:1 v/v). The solution of **L1** was then diluted to 2×10^{-5} mol L^{-1} with water/CH_3CN (1:1 v/v). In titration experiments, each time a 2×10^{-3} L solution of **L1** (2×10^{-5} mol L^{-1}) was filled in a quartz optical cell of 1 cm optical path length, and the Cu^{2+} stock solution was added into the quartz optical cell gradually by using a micro-pippet. Spectral data were recorded at 2 min after the addition. In selectivity experiments, the test samples were prepared by placing appropriate amounts of metal ion stock into 2 mL solution of **L1** (2×10^{-5} mol L^{-1}). For fluorescence measurements, excitation was provided at 495 nm, and emission was collected from 508 to 650 nm.

Fluorescent pictures were taken on Zeiss Leica inverted epi-fluorescence /reflectance laser scanning confocal microscope. Excitation of 1-loaded cells at 515 nm was carried out with a HeNe laser. Emission was collected using a 560 nm long-pass filter. Emission was collected from 570 to 625 nm. Before the experiments, cells were washed with PBS buffer and then incubated with 2×10^{-5} mol L^{-1} **L1** in DMF-PBS (1:49, v/v) for 5 h at 37 °C. Cell imaging was then carried out after washing cells with PBS.

3. Results and discussions

Recently, Duan et al. reported that a rhodamine 6 G derivative bearing a pyridine unit was capable of serving as a chemosensor for Hg^{2+}. The sensor responds to Hg^{2+} stoichiometrically, rapidly, and reversibly at room temperature through chelating with Hg^{2+} via its

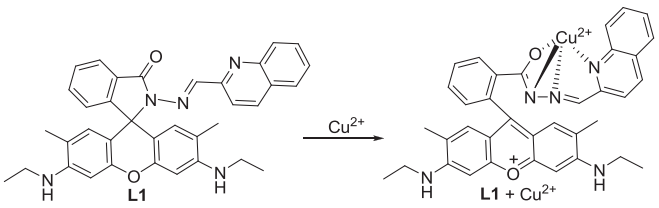

Fig. 4. Probable Complexation Mechanism of **L1**with Cu^{2+}.

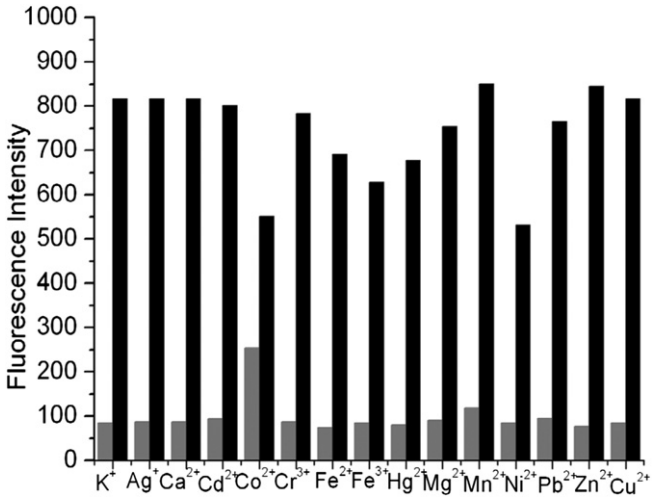

Fig. 5. Fluorescence intensities of 2×10^{-5} mol L^{-1} **L1** upon the addition of various metal ions in water/CH_3CN (1:1, v/v) solution. Black bars represent the fluorescence response of **L1** to the metal ion of interest (5 mm for K^+, Ag^+ and Ca^{2+}; 4×10^{-5} mol L^{-1} for other metal ions). Gray bars represent the addition of Cu^{2+} (4×10^{-5} mol L^{-1}) to the solution. ($\lambda_{ex} = 500$ nm).

imino N and pyridine N atoms [2e]. Herein, we report a new rhodamine derivative (**L1**) bearing a quinoline unit was served as a Cu^{2+}-selective fluorescent chemosensor and its binding mechanism toward Cu^{2+} was studied by fluorescence and absorbance spectroscopy. Compound **L1** was facilely synthesized from rhodamine 6 G by a two-step reaction in a yield of 75%. The structure of **L1** was confirmed by ^1H NMR, ^{13}C NMR, MS, and X-ray analysis (Figs. S5–7). The single crystal of **L1** was grown in trichloromethane, and an unique spirolactam structure ring formation was observed (Fig. 1).

The solution of **L1** in water/CH_3CN (1:1 v/v) is colorless and weakly fluorescent, indicating that the spirolactam form of **L1** predominantly existed under this condition. The characteristic peak of the 18-carbon of **L1** near 66 ppm in the ^{13}C NMR spectrum (Fig. S6) also supports this consideration. On the other hand, no obvious fluorescence emission of **L1** was observed between pH 5 and 12, suggesting that the compound is insensitive to pH and that the spirolactam form is still preferred in this condition. However, the addition of Cu^{2+} led to the fluorescence enhancement over a comparatively wide pH range (5.0–10.0), which is attributed to opening of the rhodamine ring (Fig. S3). These data suggest that **L1** could act as a fluorescent probe for Cu^{2+} under physiological pH conditions.

The absorption spectra of **L1** (2×10^{-5} mol L^{-1}) in water/CH_3CN (1:1 v/v) exhibited only a very weak band above 500 nm, which was ascribed to the spirolactam form of molecule **L1**. Upon addition of Cu^{2+} to a solution of **L1**, the solution turned from colorless to pink, and the absorbance was significantly enhanced (>150-fold) and a new peak at 531 nm was observed (Fig. 2a), clearly suggesting the formation of the ring-opened amide form of **L1** upon Cu^{2+} binding [10]. Other metal ions, such as K^+, Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+} did not show any significant color and spectral change under identical conditions (Fig. 3).

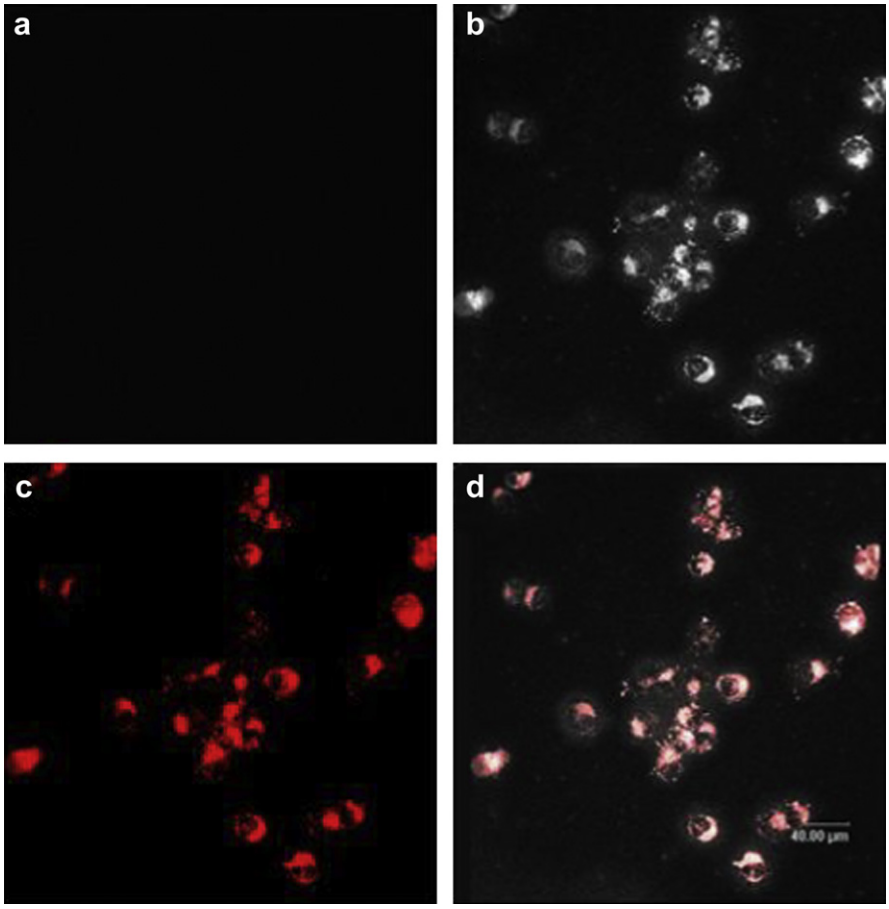

Fig. 6. Confocal fluorescence, brightfield, and overlay images of EJ (cystic cancer) cells. a) Fluorescence image of EJ cells treated with L1 (2×10^{-5} mol L^{-1}) in the absence of $CuCl_2$ (control). b) Brightfield image of cells shown in panel c. c) Cells supplemented with 2×10^{-5} mol L^{-1} **L1** in the growth media for 5 h at 37 °C and then incubated with 40 µm $CuCl_2$ for 0.5 h at 25 °C. The overlay image of b) and c) is shown in d) ($\lambda_{ex} = 488$ nm).

The sensing behavior was investigated by the fluorescence measurement in water/CH_3CN (1:1 v/v) upon excitation at 500 nm. As shown in Fig. 2b, a sharp decrease in the fluorescence intensity at 550 nm is observed on addition and gradual increase of [Cu^{2+}] ($0-5\times10^{-5}$ mol L^{-1}). The association constant for Cu^{2+} was estimated to be 4.03×10^{3} M^{-1} in the water/CH_3CN (1:1, v/v) solution on the basis of linear fitting of the titration curve assuming a 1:1 stoichiometry (Fig. S1). This binding mode was also supported by a Job plot (Fig. 2b inset). Moreover, this binding mode is supported by the presence of a peak at $m/z = 630.3$ (calcd = 630.23) corresponding to [**L1** + Cu]$^+$ in the ESI-MS spectrum of a mixture of **L1** and 1 equiv $CuCl_2$ (Fig. S8).

Thus, in accordance with the 1:1 stoichiometry, the sensors are the most likely to chelate with metal ions via its carbonyl O, imino N, and Quinoline N atoms (Fig. 4).

To validate the selectivity of **L1** in practice, some other cations were added to a solution of **L1** under the sameconditions. As shown in Fig. 5, only Co^{2+} induced a mild fluorescence intensity enhancement, while the other metal ions did not cause any discernible changes. Moreover, these co-existent ions had negligible interfering effect on Cu^{2+} sensing by **L1**, even when K^+, Ag^+, and Ca^{2+} were present at micromolar levels. All of these results indicate the high selectivity of **L1** for Cu^{2+} over other competing cations in water/CH_3CN (1:1 v/v) solution.

We also investigated the time course of the response of **L1** to 5 equiv of Cu^{2+} in water/CH_3CN (1:1, v/v). We found that the interaction of **L1** with Cu^{2+} was completed in less than 2 min. Therefore, this system could be used for real-time tracking of Cu^{2+} in cells and organisms (Fig. S3).

To further demonstrate the practical application of the probe, we carried out experiments in living cells. EJ (lung cancer) cells were incubated with 2×10^{-5} mol L^{-1} **L1** for 5 h at 37 °C, and very weak fluorescence of **L1** inside the living EJ cells was observed (Fig. 6a). After three times washing with PBS buffer, the cells were incubated with Cu^{2+} (40 μM) in the medium for another 0.5 h at 37 °C, and the fluorescence in living cells was much brighter (Fig. 6c). Bright-field measurements after treatment with Cu^{2+} and **L1** confirmed that the cells were viable throughout the imaging experiments (Fig. 6b). As depicted in Fig. 6d, the overlay of fluorescence and bright-field images reveals that the fluorescence signals are localized in the perinuclear area of the cytosol, indicating a subcellular distribution of Cu^{2+}. These results demonstrate that **L1** is cell-permeable and primarily nontoxic to the cell culture.

4. Summary

In summary, we have synthesized a new rhodamine-based chemosensor **L1**, which displayed an excellent selectivity for Cu^{2+} over other metal ions examined in water/CH_3CN (1:1, v/v) solutions. Upon the addition of Cu^{2+}, the spirolactam ring of **L1** was opened and a 1:1 metal-ligand complex was formed. Moreover, fluorescence imaging for Cu^{2+} in living cells shows **L1** has ideal chemical and spectroscopic properties that satisfy the criteria for further biological applications.

Acknowledgements

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Appendix. Supplementary data

Experimental procedures for the synthesis, spectral data, and copies of ^1H NMR and ^{13}C NMR of **L1**, data for UV–vis, fluorescence of **L1**, and other data are available. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.dyepig.2011.01.003.

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