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A selective chemosensor based on rhodamine B hydrazone for Cu(II)

Zhiyu Ju [1,*], Zhihong Xu [1], Changzeng Wu [1]

[1]College of Chemistry and Chemical Engineering, Xuchang University, Xuchang 461000, P. R. China

*corresponding author e-mail address: ***juz1696@163.com***

ABSTRACT

A rhodamine-based fluorescent probe was synthesized using quinoline moiety, and the sensitive and selective detection of Cu^{2+} ion was investigated by absorption and emission spectroscopy. The optical properties of this compound have been investigated in PBS buffer containing CH_3CN/H_2O (7:3 v/v) solution.

Keywords: *Fluorescent probes, Rhodamine B, Cations, Quinoline*.

1. INTRODUCTION

The design and synthesis of fluorescent artificial chemosensors for selective and sensitive quantification of biologically and environmentally heavy- and transition-metal ions in solution has attracted widely interests of chemists, biologists, clinical biochemists and environmental scientists [1]. Various transition-metal ions are crucial for the life of organisms. Among these ions, the copper ion, which plays a critical role as a catalytic cofactor for a variety of metalloenzymes. However, under overloading conditions, copper exhibits toxicity in that it causes neurodegenerative diseases [2]. Thus, the quantitative detection of Cu(II) is of great importance for elucidating its complex physiological and pathological roles. Screening methods for copper have atomic absorption spectrometry (AAS) [3], inductively coupled plasma mass spectrometry(ICP-MS) [4], and inductively coupled plasma atomic emission spectrometry (ICP-AES) [5], often require expensive and sophisticated instrumentation or complex sample preparation steps. Chemosensors based on the Cu(II)-induced changes in fluorescence would be more desirable, because it is less labor-intensive and highly sensitive. Generally, the typical chemosensor is constructed by covalent linkage of three parts, namely, a receptor unit, a spacer and a signaling unit, which displays completely different absorption/fluorescence signals compared to free chemosensors in solution after binding with metal ions which enables the quantitative determination of Cu(II) ions [6].

However, the fluorescence enhancement or quenching based on rhodamine derivatives in the presence of Cu^{2+} occurring in the longer absorption or emission wavelength region has rarely been reported. In our previous work, rhodamine B hydrazide exhibited an irreversible colorimetric and fluorogenic response toward Cu^{2+} in aqueous solution in the red region [7]. The longer emission wavelengths (over 550 nm) are often preferred for the analyte, to avoid the influence of background fluorescence (below 500 nm) [8]. As the absorption enhancement of a host in the presence of guests in the red region facilitates naked-eye recognition, spirolactam forms of rhodamine B hydrazone **RQ** is synthesized to develop reversible Cu^{2+} chemosensor in the red region in aqueous solutions. The opening of the spirolactam forms may be realized by the interaction of the Cu^{2+} with the corresponding N and O atoms in these compounds.

2. EXPERIMENTAL SECTION

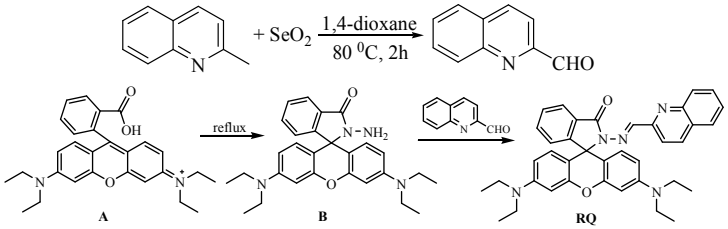
2.1. General experiments. All reagents were used as purchased without any purification. NMR experiments were performed with a Bruker AV 400 spectrometer and the chemical shifts were recorded with respect to TMS as an internal reference. UV–Vis and fluorescence experiments were carried out by Shimadzu UV-2550 spectrophotometer (Tokyo, Japan) and Hitachi F-4600 spectrofluorimeter, respectively. The salts of Al^{3+}, Co^{2+}, Ni^{2+}, Hg^{2+}, Zn^{2+}, Cd^{2+}, Cr^{3+}, Fe^{3+}, Mg^{2+}, Ca^{2+}, Li^{+}, Na^{+}, K^{+}, and Ag^{+} ions were used and stock solutions of **RQ** was prepared in CH_3CN and water solution (PBS: 20 mM).

2.2. Procedures of metal ion sensing. Nitrate salts of metal ions were used to evaluate the metal ion binding property and selectivity of compound **RQ** in PBS buffer containing CH_3CN/H_2O (7:3 v/v) solution at pH 7. Stock solutions of the metal ions (5 mmol/L) were prepared in de-ionized water. In titration experiments, 3 mL solution of compound **RQ**, which was diluted to a certain concentration with PBS buffer containing CH_3CN/H_2O (7:3 v/v) solution at pH 7, was added into a quartz optical cell with an optical path length of 1 cm. The stock solution of each metal ion was added into the quartz optical cell step by step via a syringe. The spectra were recorded at 1 min after the addition and mixing. For fluorescence measurements, excitation wavelength was provided at 520 nm, and emission was collected from 530 to 720 nm.

2.3. Synthesis

2.3.1. Synthesis of Quinoline-2-carbaldehyde. A mixture of 4.7 g of selenium dioxyde (42 mmol) in 20 mL dioxane and 8 ml water was added in small portions over 10 minutes to a boiling solution of 2 g (14 mmol) of 2-methylquinoline in 20 mL dioxane. After 6 hours of boiling under nitrogen, the warm reaction mixture was cooled and filtered. The filtrate was evaporated, dissolved in dichloromethane and filtered through silica gel. The yellow-brown

solid product obtained after evaporation of the solvent was recrystallized from dichlormethane. Yield: 3.76 g (78 %). ^{1}H NMR (400 MHz, DMSO) δ 10.14 (s, 1H), 8.61 (d, *J* = 8.4 Hz, 1H), 8.24 (d, *J* = 8.5 Hz, 1H), 8.13 (d, *J* = 8.2 Hz, 1H), 7.95 (t, *J* = 17.2, 9.7, 4.9 Hz, 2H), 7.85–7.75 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 194.28, 152.84, 147.67, 138.49, 131.35, 130.28, 130.14, 129.84, 128.75, 117.61.



Scheme 1. Synthesis of Quinoline-2-carbaldehyde and compound **RQ**.

2.3.2. **Synthesis of compound RQ.** Compound B was synthesized using a modified procedure from Yang et al [9]. The compound RQ was synthesized as shown in Scheme 1, 0.912 g (0.002 mol) of compound B and 0.488 g (0.0031 mol) of quinoline-2-carbaldehyde were dissolved in 15 mL of anhydrous ethanol. The mixture was refluxed under N_2 for 10 h. The cooled solution was partially removed upon rotary evaporation. The resulting solid was further purified by column chromatography to afford a pale-yellow solid (0.774 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.13 (d, *J* = 8.7 Hz, 1H), 8.01 (dd, *J* = 16.5, 8.2 Hz, 3H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.62 (t, *J* = 7.7 Hz, 1H), 7.49 (ddd, *J* = 18.3, 11.0, 6.8 Hz, 3H), 7.14 (d, *J* = 7.3 Hz, 1H), 6.56 (t, *J* = 6.1 Hz, 2H), 6.48 (dd, *J* = 9.6, 6.8 Hz, 2H), 6.24 (dd, *J* = 8.9, 2.6 Hz, 2H), 3.31 (q, *J* = 7.0 Hz, 8H), 1.14 (t, *J* = 7.0 Hz, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.45, 155.09, 153.10, 152.31, 149.03, 147.77, 146.75, 135.81, 133.77, 129.37, 129.25, 128.32, 128.23, 127.68, 127.56, 126.77, 123.82, 123.68, 118.36, 108.06, 105.83, 98.27, 66.13, 44.33, 12.64. ESI-MS *m/z*: Calcd for $C_{38}H_{38}N_5O_2^+$ $[M+H]^+$ 596.3, found: 596.5.

3. RESULTS SECTION

3.1. UV–Vis Spectral Responses of RQ. Compound RQ was proposed to chelate with metal ions via its carbonyl O, imino N, and quino N, atoms. The introduction of the rhodamine framework to construct probes of the "off-on" type was a reliable method due to the well-known spirolactam ("off") to ring-opened amide ("on") equilibrium of rhodamine derivatives. The spirolactam moiety of the rhodamine framework served as a signal switcher, which was observed to turn on when the cation was bound.[16] The solution of RQ was almost colorless and exhibited almost no absorption in the visible wavelength range (400-650 nm) (Fig. 1), indicating that RQ is predominantly in the form of spirolactam. The same observation prevails when different metal ions (e.g. Al^{3+}, Co^{2+}, Ni^{2+}, Hg^{2+}, Zn^{2+}, Cd^{2+}, Cr^{3+}, Fe^{3+}, Mg^{2+}, Ca^{2+}, Li^+, Na^+, K^+, and Ag^+) were added to the solution of RQ in Fig. 1. However, there was only a remarkable enhancement of absorption in the range of 465–600 nm upon addition of Cu^{2+} to the solution of RQ, along with an obvious color change from colorless to purple. Also, it was investigated that the fluorescence response of compound RQ toward Cu^{2+} in the presence response of various coexistent ions. It is gratifying to note that all the tested ions have no interference (Fig. S6).

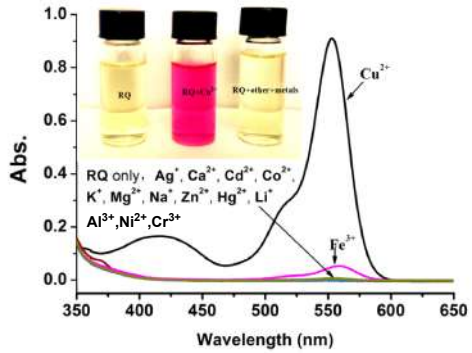


Figure 1. Absorbance spectra of RQ (10 μM) in the absence and presence of 10 eq. different metal ions in CH_3CN/PBS (20 mM, pH=7.0, 7:3, v/v) solution. Insert shows the photo of probe RQ with different metal ions.

These results demonstrated that RQ can be served as a"naked eye"probe with a characteristic of high selectivity toward Cu^{2+} over other competitive metal ions.

To further investigate the interaction of Cu^{2+} and RQ, an ultraviolet titration experiment was carried out. As shown in Fig. 2, upon addition of Cu^{2+}, a new absorption peak at 553 nm appeared, and the absorption intensity increased gradually with increasing of Cu^{2+} concentration. The changes in the absorption spectra of RQ upon addition of Cu^{2+} indicated the obvious interaction of Cu^{2+} and compound RQ. Nonlinear fitting based on the 1:1 stoichiometry (insert in Fig. 2) indicated a very strong binding ability of RQ to Cu^{2+}. A Job's plot (Fig. 3) analysis corresponding to 0.50 the concentration ratio indicates the 1:1 stoichiometric ratio between RQ and Cu^{2+}.

3.2. Fluorescence Spectral Responses of RQ. The selectivity of RQ for Cu^{2+} was further observed in the fluorescent spectra. As shown in Fig. 4, compound RQ exhibited a very weak fluorescence in the absence of metal ions.

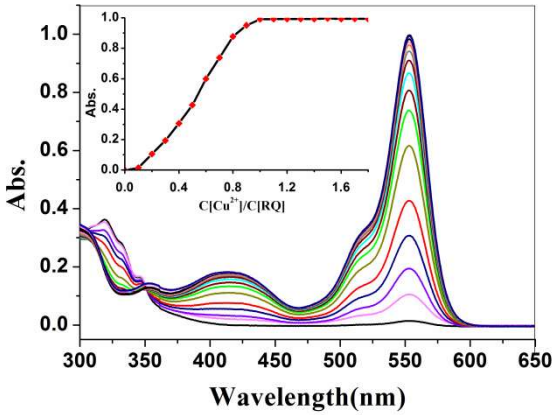


Figure 2. Absorption spectra of RQ (10 μM) with gradual addition of various amounts of Cu^{2+} in CH_3CN/PBS (20 mM, pH=7.0, 7:3, v/v) solution. Insert shows the titration profile evaluated from the absorption intensity at 553 nm.

When 5 equiv. Cu^{2+} was introduced in a 10 μM solution of RQ in CH_3CN/PBS (20 mM, pH=7.0, 7:3, v/v) solution, a remarkably enhancement of fluorescence spectra was observed.

Competition experiments were carried out to explore the use of RQ as an ion-selective fluorescent probe for Cu^{2+}. RQ (10 uM) was treated with 5 equiv. Cu^{2+} in the presence of other metal ions (5 equiv.). Moreover, the color of the solution changed from a blue state to yellow color under UV illumination (365 nm) (Fig. 4 inset). The results demonstrated that RQ was characteristic of high selectivity toward Cu^{2+} over other competitive metal ions.

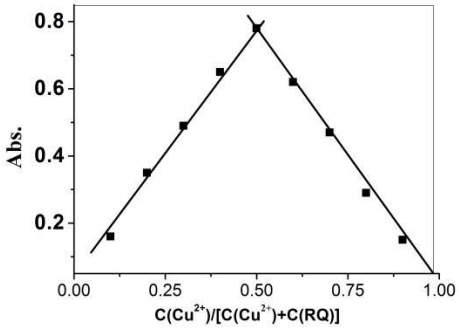


Figure 3. Job's plot for the complex of RQ and Cu^{2+} at 562 nm. Total concentration of [RQ]+[Cu^{2+}] was kept constant at 10 μM.

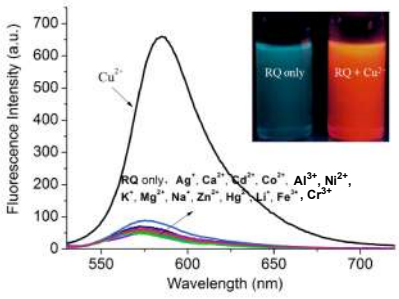


Figure 4. Fluorescence spectra of RQ (10 μM) in the presence of 50 μM different metal ions in CH_3CN/PBS (20 mM, pH=7.0, 7:3, v/v) solution. λ_{ex} = 520 nm, scan range 530–720 nm, slit width 5 nm. Inset: fluorogenic response of RQ (10 M) in CH_3CN/PBS (20 mM, pH=7.0, 7:3, v/v) solution to Cu^{2+} (5 equiv.) under UV illumination (365 nm).

Besides achieving high selectivity toward specific analyst over other competitive species is an important feature for an excellent sensor. Therefore, the competitive experiments were extended to miscellaneous metal ions to indicate the selectivity of the fluorescence probe. As shown in Fig. 5, since the addition of Cu^{2+} ions, an obvious enhancement in fluorescence intensity at 572 nm was observed. These results indicated that the background metal ions showed small or no interference with the detection of Cu^{2+} ion in CH_3CN/PBS (20 mM, pH=7.0, 7:3, v/v) solution.

The fluorescence titration of Cu^{2+} ion was performed with RQ (10 μM) in CH_3CN/PBS (20 mM, pH=7.0, 7:3, v/v) solution. Upon the addition amount of Cu^{2+} (0-10 eq.), the fluorescence emission intensity at 572 nm gradually increased to the maximum at 5.0 eq., and after 5.0 eq. (excess), the emission intensity became constant (Fig. S7). However, the adding of Cu^{2+} caused a red-shift with a decrease of the maximum emission at 572 nm.

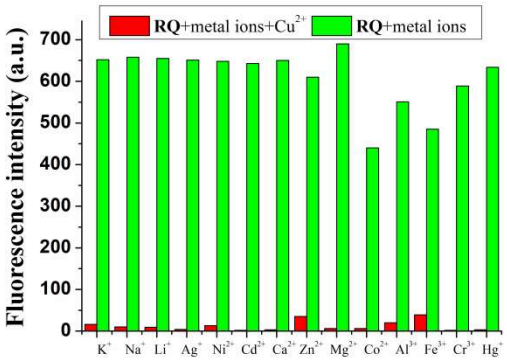


Figure 5. Fluorescence responses of RQ (10 μM) to various cations in CH_3CN/PBS (20 mM, pH=7.0, 7:3, v/v) solution. λ_{ex} = 520 nm, λ_{em} =572 nm.

To investigate the practical applicability of RQ, the detection limit of this new probe system was evaluated. The linearity via plotting the fluorescence emission intensity of RQ at 572 nm as a function of the Cu^{2+} concentration under arrange of 0–10 μM with a correlation coefficient of 0.98757(Fig. 6). Based on $3\delta/k$ (where δ is the standard deviation of the blank solution and k is the slope of the plot), the limit of detection for Cu^{2+} was up to 0.128 μM [10]. These results revealed that the probe RQ was sensitive and selective toward the detection of Cu^{2+} by the fluorescence spectra.

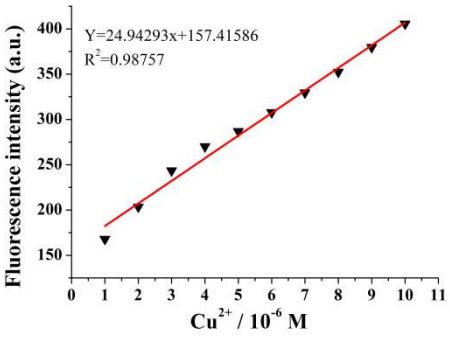


Figure 6. The fluorescence intensity (at 572 nm) of compound RQ (10 μM) as a function of the Cu^{2+} concentration (1–10 μM) in in CH_3CN/PBS (20 mM, pH=7.0, 7:3, v/v) solution (λ_{ex} = 520 nm, slit width 5 nm).

3.3. Mechanism. All of the above absorption spectra responses were reversible, which was confirmed by the reversible titration using EDTA. When excess EDTA (2 equiv. of Cu^{2+}) was added to the RQ + Cu^{2+} in (6:4, v/v) CH_3CN/water solution (20 mM PBS, pH = 7.0), the red color of the solution changed, indicating that the coordination of RQ with Cu^{2+} was chemically reversible. And the fluorescence emission changes of above solution had also confirmed it. Thus, based on the 1:1 binding mode and the reversible behavior between RQ and Cu^{2+}, and according to our knowledge, we broached a conceivable mechanism of Cu^{2+} complex with RQ (Scheme 2). A directly evidence was obtained by comparing the ESI-MS of RQ (m/z=595.5) and RQ + Cu^{2+} (m/z=659.2) in (6:4, v/v) CH_3CN/water solution (20 mM PBS, pH = 7.0).

4. CONCLUSIONS

In summary, we have developed a rhodamine B-based chemosensor bearing xanthenes and quinoline group for the detection of Cu^{2+}. Compound RQ displayed selective fluorescent and colorimetric changes upon addition of Cu^{2+}. Compound RQ was found to be an excellent sensor for Cu^{2+} using either of the two very modes of measurements (absorption and emission). The significant changes in the fluorescence color could be used for naked-eye detection.

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6. ACKNOWLEDGEMENTS

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