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ARTICLE

High sensitive and selective turn-on fluorescent probe for Cu^{2+} based on Rhodamine B

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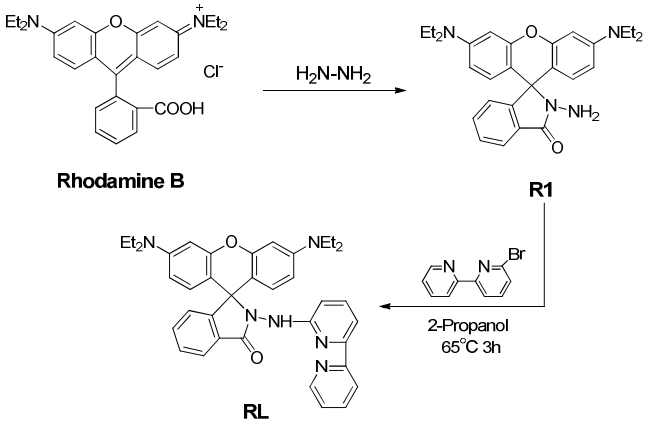
A new rhodamine B-based fluorescent probe RL for Cu^{2+} has been designed, synthesized, and characterized. It exhibits a very high selectivity and sensitivity for the detection of Cu^{2+} in CH_3CN：H_2O = 1：9 (v/v) buffered with Britton-Robinson (pH = 7.02), the detection limit can reach 0.739 nM, which is the lowest reported. Furthermore, the probe RL can be made into test papers to detect the cupric ions in drinking water by naked eyes. RL can also be used for imaging of Cu^{2+} in living cells and is almost non-toxic.

Introduction

As the third most abundant essential trace element, Copper plays an important role in biological process, because of its redox reactivity, however, this could also do harm to the organisms.[1-3] Copper deficiency may lead to some serious diseases, such as Alzheimer's,[4] Menkes' and Wilson's,[4] amyotrophic lateral sclerosis,[5] and prion diseases.[6] Thus, developing a simple and rapid method to detect copper ions is particularly important, which would make a contribution to the understanding of these diseases aroused by copper ions.

In recent years, fluorescent chemosensors have been widely used to detect metal ions owing to their high sensitivity, good selectivity, short response time and real-time monitoring etc. One of the most commonly used dye model is rhodamine B, because it has excellent photophysical properties, such as long absorption and emission wavelengths extended to visible region, high fluorescence quantum yield, and large extinction coefficient etc.[7] Many fluorescent chemosensors based on rhodamine B and its derivatives have been developed for Hg^{2+},[8-10] Zn^{2+},[11,12] Cu^{2+},[1,13-15] Pd^{2+},[16,17] Fe^{3+} [18,19], H_2S[20], DNA[21-24]etc. For example, Dujols[25] firstly used rhodamine B hydrazide as a chemodosimeter for Cu^{2+} detection. Kim and coworkers[26] synthesized a novel ratiometric and selective fluorescent sensor for Cu^{2+} based on two different approaches. Iyer[27] developed a highly selective probe to detect Cu^{2+} and endogenous NO gas in living cell. Lin etc.[28] developed a FRET-Based ratiometric fluorescent Cu^{2+} chemodosimeter, which had a good application for living cell imaging. Kumar[29] synthesized a highly selective fluorescence turn-on chemodosimeter based on rhodamine for nanomolar detection of Cu^{2+}. Peng etc.[30] synthesized a fluorescent ratiometric chemodosimeter for Cu^{2+} based on TBET with its application in living cells. Lin[31] synthesized a novel pyrene- and anthracene-based schiff base derivatives as Cu^{2+} and Fe^{3+} fluorescence turn-on sensors with



Scheme 1. Synthesis and structures of the rhodamine B derivative chemosensor RL

aggregation induced emissions. Kim and coworkers[32] synthesized a novel ratiometric and selective fluorescent sensor for Cu^{2+} based on two different approaches. Peng etc.[33] synthesized a series of highly sensitive and selective fluorescent and colorimetric "off-on" chemosensors for Cu (II) based on rhodamine derivatives. Tian[34] synthesized a hydrophilic copolymer as fluorescent film sensor for Cu^{2+} and pyrophosphate anion. Goswami[35] developed a new highly selective, ratiometric and colorimetric fluorescence sensor for Cu^{2+} with a remarkable red shift in absorption and emission spectra based on internal charge transfer. Although significant progress has been achieved in related field, further study is still needed to design more efficient and practical sensors to monitor Cu^{2+} under different circumstance, for instance, the Cu^{2+} concentration in drinking water.

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Herein, we designed and synthesized a new rhodamine B-based fluorescent probe RL (Scheme 1) for Cu^{2+} detection, which can be employed in CH_3CN: H_2O = 1: 9 (v/v) buffered with Britton-Robinson (pH = 7.02), and the detection limit can reach 0.739 nM, this is the lowest one reported till now. To check its applicability, the probe RL was made into test papers to detect the cupric ions in drinking water; the detection limit reaches 10 μM (0.64 mg/L) by naked eyes, which is lower than the permissible Cu^{2+} concentration in drinking water that the World Health Organization had claimed (1 mg/L). So the probe can be employed to detect the cupric ions in drinking water. Furthermore, RL can work pretty well for imaging of Cu^{2+} in living cells and is almost non-toxic.

Experimental

Materials and Apparatus

All reagents and solvents were purchased from commercial sources, and the solvents used were of analytical grade. Doubly purified water used in all experiments was from Milli-Q systems. The RL stock solution was prepared at a concentration of 1.0×10^{-3} M in 10 mL DMSO, and the stock solution was diluted to a desired concentration for each titration in a 3 mL cuvette using CH_3CN: H_2O = 1: 9 (v/v) buffered with Britton-Robinson, pH = 7.02. Each inorganic metal salt and anions stock solutions were prepared at the concentration of 25.0 mM, the concentration of $CuCl_2$ is 5.0 mM. The ^1H NMR and ^{13}C NMR spectra were recorded on a VARIAN INOVA-400 spectrometer. Mass spectrometric data were obtained at Q-Tof MS spectrometer (Micromass, Manchester, England). Fluorescence spectra were obtained by Agilent Cary Eclipse fluorescence spectrophotometer. Absorption spectra were obtained by Agilent 8453 UV-Visible spectrophotometer. The pH values were measured with a Model pHS-3C pH meter (Shanghai, China). Fluorescence imaging was performed using an OLYMPUS FV-1000 inverted fluorescence microscope with a 60×objective lens.

Synthetic procedures

Synthesis of R1

R1 was synthesized according to the literature.[25]

Synthesis of RL

R1 (0.5 g, 1.00 mmol) was dissolved in 35 mL 2-propanol in a 100 mL flask, then 2.33 g (10.0 mmol) 6-bromo-2,2'-bipyridine was added to the flask under vigorous stirring. The reaction mixture was heated to 65 ºC for 3 h, then the reaction was cooled to room temperature and washed with saturated $NaHCO_3$, the concentrated crude compound was purified by column chromatography on silica gel (60:1 dichloromethane : methanol v/v) to afford a pink solid (520 mg, 79 %). ^1H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 4.6 Hz, 1H), 8.19 (d, J = 8.0 Hz, 1H), 8.09 – 7.96 (m, 1H), 7.78 – 7.51 (m, 4H), 7.32 (t, J = 7.9 Hz, 1H), 7.24-7.19 (m, 1H), 6.54 (d, J = 9.2 Hz, 2H), 6.30 (d, J = 8.1 Hz, 4H), 6.14 (s, 1H), 3.30 (q, J = 7.0 Hz, 8H), 1.13 (t, J = 7.0 Hz, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.57, 158.31, 153.88, 150.38, 148.79, 138.05, 136.57, 133.24, 130.41, 128.57, 124.46, 123.27, 121.48, 113.26, 107.71, 98.00, 66.41, 44.39, 12.47. HRMS: $[M+Na]^+$, calcd: m/z = 633.2954, found: m/z = 633.2941

Results and discussion

Fluorescence response time of RL with Cu^{2+}

Changes in the fluorescence intensity (I_{582} nm) of RL on treatment with $CuCl_2$ were monitored over time in Figure 1. It can be observed that the fluorescence intensity increased rapidly after the addition of Cu^{2+}, and reached a maximum in 10 min. Therefore, all the subsequent fluorescent measurements were carried out after allowing for a 10 min equilibration time.

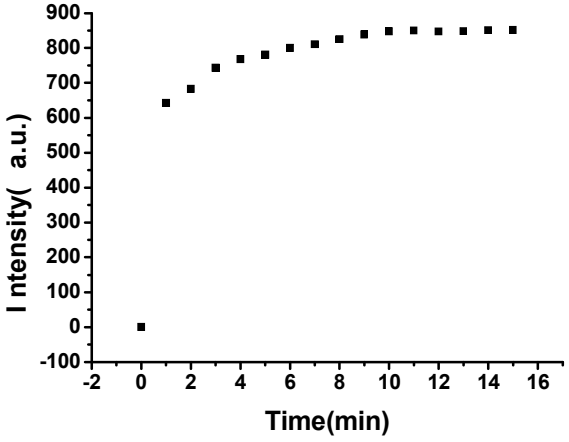

Figure 1. Time-dependent fluorescent intensities (I_{582} nm) change of RL (10 μM) with $CuCl_2$ (10 μM) in CH_3CN: H_2O = 1:9 (v/v) buffered with Britton-Robinson, pH = 7.02, λ_{ex} = 540 nm

Fluorescence and UV-vis Spectra Titration of RL

The change in fluorescence and absorption spectra of RL upon titration with $CuCl_2$ are displayed in Figure 2. In the presence of $CuCl_2$, RL (10 μM) in the CH_3CN/H_2O (1:9, v/v) buffered with Britton-Robinson (pH = 7.02) displayed a fluorescence peak at 582 nm. With continuous addition of $CuCl_2$, the absorption maximum changed from 575 nm to 550 nm and the absorbance value increased from 0 to 0.46, together with an obvious color change to pink. According to the result of titration experiment (Figure 3), the detection limit was 0.739 nM,[26] which was the lowest that had been ever reported.

Fluorescence properties

The high selectivity of RL for Cu^{2+} was further evaluated by the fluorescent spectra. As shown in Figure 4, no fluorescence can be observed when 10 equiv. metal ions of Pb^{2+}, Fe^{3+}, Ag^+, Ca^{2+}, Cd^{2+}, K^+, Co^{2+}, Na^+, Cr^{3+}, Fe^{2+}, Hg^{2+}, Al^{3+}, Mn^{2+}, Ni^{2+}, Zn^{2+} were added and measured 10 minutes later, only Cu^{2+} ions induced a large increase in fluorescence intensity of RL.

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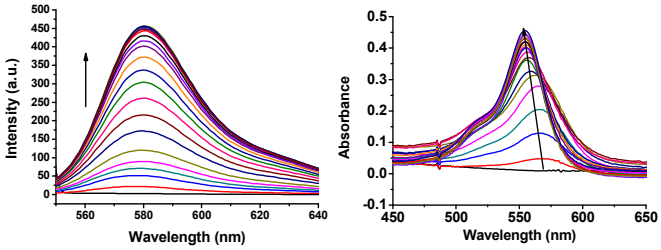

Figure 2. The fluorescence and absorption (λ_{ex}=540 nm) spectra of RL (10 μM) along with the addition of $CuCl_2$ (0– 40 μM) in CH_3CN/H_2O (1:9, v/v) buffered with Britton-Robinson, at room temperature. pH = 7.02. All spectra were recorded 10min after the addition of $CuCl_2$.

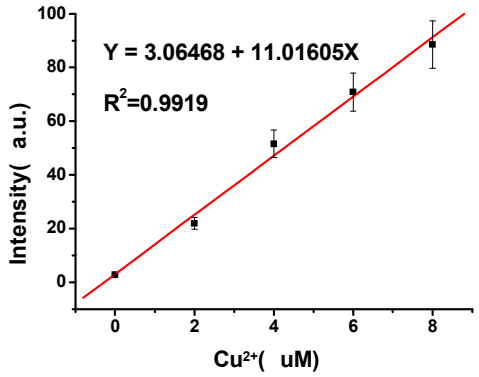

Figure 3. Fluorescent intensities change of RL (10 μM) in the presence of different concentrations of $CuCl_2$ (0 – 8 μM).

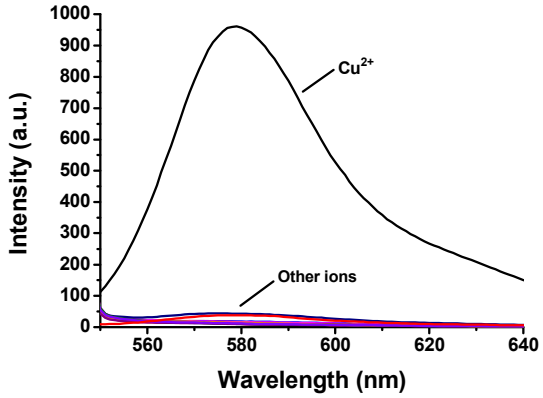

Figure 4. Fluorescence intensity of RL (10 μM) in the presence of different metal ions (30 μM for Cu^{2+} ions and 100 μM for other metals ions) in CH_3CN/H_2O (1:9, v/v) buffered with Britton-Robinson, pH = 7.02. λ_{ex} = 540 nm.

To investigate the influence of the different metal ions, interference experiments (determining Cu^{2+} ions in the presence of other ions) were also performed (Figure 5, Figure 6). Most of the metal ions caused only little variations in the fluorescence intensity when compared with that obtained in the absence of any interference ions, and not any obvious reducing of the fluorescence intensity was observed except Pb^{2+}、Hg^{2+}、SO_3^{2-} ions. This suggested the probe can be used to detect Cu^{2+} ions in complex environment.

Effect of pH

The spirolactone ring of the rhodamine moiety in RL is susceptible to change with different pH; typically at acidic pH, the ring opens, making the non-fluorescent rhodamine moiety emit red fluorescence. Such a behavior can interfere with the detection of Cu^{2+} ions. Therefore, we evaluated the fluorescence properties of RL in solutions with different pH values (2.66–7.02, Figure 7). The fluorescence intensity remained stable in the pH range 5.3-7.0, but it increased as the

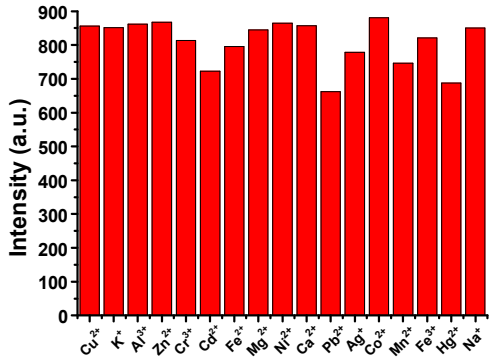

Figure 5. Fluorescence intensity of RL (10 μM) after addition of $CuCl_2$ (30 μM) in the presence of other metal ions (100 μM) in CH_3CN/H_2O (1:9, v/v) buffered with Britton-Robinson, pH = 7.02. λ_{ex} = 540 nm.

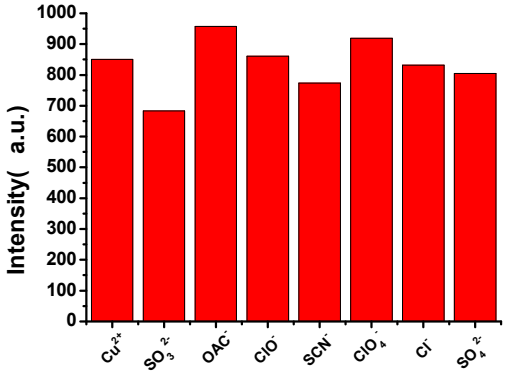

Figure 6. Fluorescence intensity of RL (10 μM) after addition of $CuCl_2$ (30 μM) in the presence of other common anions (100 μM) in CH_3CN/H_2O (1:9, v/v) buffered with Britton-Robinson, pH = 7.02. λ_{ex} = 540 nm.

pH values decreased from 5.3 to 2.66, which was attributed to the opening of the spirolactone ring. The analysis of the pH curve determined the pKa of RL to be 3.70.

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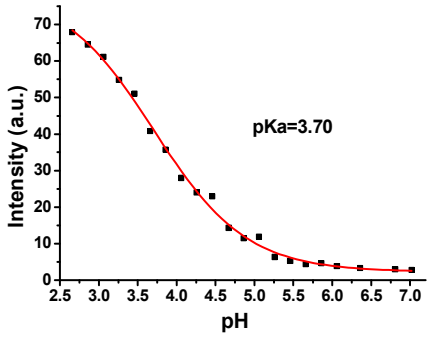
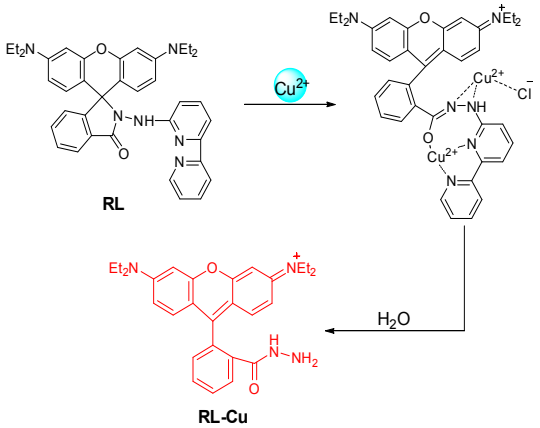


Figure 7. Plot depicting the pH (2.66–7.02)-dependent variation in the fluorescence intensity of RL (10 μM) in Britton-Robinson buffer solution. λ_{ex} = 540 nm.

Reaction mechanism

From Figure S2 we can see that the reaction between probe RL and Cu^{2+} is irreversible, so it may be a chemical change, this fluorescence enhancement response of chemosensor RL in the presence of Cu^{2+} is most likely the result of ion-catalyzed hydrolysis reaction, which can be observed in the Mass Spectra (Figure S4), the molecular ion peak of RL changes rapidly from 611.43 to 457.33 in the presence of Cu^{2+}. We did a reaction of Cu^{2+} and RL, 5 min later, TLC was employed to check the status. As shown in Figure S5(a), a new point of RL-Cu was observed, which exhibited the same R_f as R1 , and the reactant RL was still existed, this means that the hydrolysis occurred under the catalysis of Cu(II). And about 12 min later, we did the TLC experiment again, as shown in Figure S5(b), only the new point RL-Cu can be observed, and both R1 and RL-Cu are in the fluorescent ring opening form on the acidic silica gel plate. The product of RL-Cu was collected and ^{1}H NMR spectra were measured in both $CDCl_3$ (Figure S6) and DMSO-d_6 (Figure S7), not much difference can be observed compared with that of R1. Meanwhile, HRMS (Figure S8) can provide some further evidence. All these demonstrated that the hydrolysis product of RL-Cu is the ringing opening form of R1,and two Cu^{2+} ions are coordinated with one RL molecule, which could be supported by the results of the Job-plot (Figure S1).The hydrolysis mechanism can be proposed as follows:



Scheme 2. The Proposed Determination Mechanism

Fast detection of Cu^{2+} in filter papers

Filter papers were immersed in a dichloromethane solution of RL (1 mM) and then dried in the air to prepare test strips. After immersing them in the cupric ion aqueous solution for several seconds and drying in the air, the test strips exhibited a rapid color change with the increase of the Cu^{2+} concentration, which can be observed by naked eyes. From the filter papers in Figure 8, we can see the color change ranged from colorless to purple, and the detection limit reaches 10 μM (0.64 mg/L), this also is the lowest detection limit that reported in practical application and is lower than the permissible Cu^{2+} concentration in drinking water that the World Health Organization had claimed (1 mg/L). Thus, the test papers of probe RL can be used to monitor the cupric ions in drinking water.

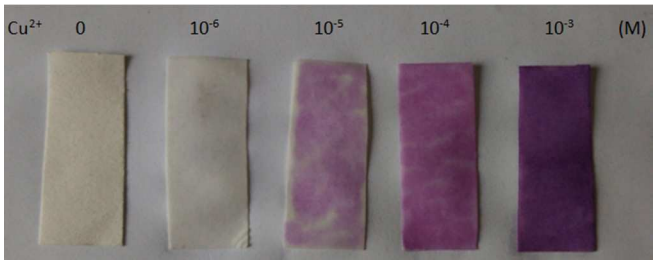


Figure 8. Color changes of paper test strips for detecting cupric ions in aqueous solution with different cupric ion concentrations. Left to right: 0, 1.0×10^{-6} M, 1.0×10^{-5} M, 1.0×10^{-4} M, 1.0×10^{-3} M.

Cell imaging

To further check the practical applicability of probe RL in biological samples, fluorescence imaging experiments were carried out in living MCF-7 cells (Figure 9). In the absence of Cu^{2+}, RL showed no detectable fluorescence signal in living cells. After incubation with Cu^{2+}, a bright fluorescence was observed in living cells, and the MTT experiments (Figure S3) indicate that probe RL is almost non-toxic. The results suggest that probe RL can be used for imaging of Cu^{2+} in living cells.

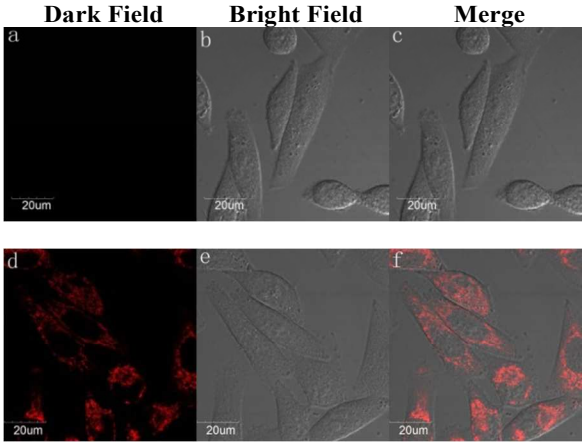


Figure 9. Fluorescence imaging of RL (10 μM) in MCF-7 cells in the absence (a) and presence (d) of Cu^{2+} ion (50 μM). λ_{ex} =

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515 nm.

Conclusion

In summary, a highly selective and sensitive fluorescent probe RL for Cu^{2+} ions was synthesized and characterized. The probe RL exhibited colorimetric responses to Cu^{2+} ions, providing a method for visual detection of Cu^{2+} ions. This probe can work in CH_3CN：H_2O = 1：9 (v/v) buffered with Britton-Robinson (pH = 7.02) with a detection limit of 0.739 nM, which is the lowest ever reported till now. The probe RL can be made into test papers to detect the cupric ions in drinking water with the real detection limit of 10 μM (0.64 mg/L), which is lower than the permissible Cu^{2+} concentration in drinking water that the World Health Organization had claimed (1 mg/L). Furthermore, the probe RL can be used for imaging of Cu^{2+} in living cells and is almost non-toxic.

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Notes and references

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Electronic Supplementary Information (ESI) available: ^{1}H NMR, ^{13}C NMR, and MS. See DOI: 10.1039/b000000x/

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