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Characterization of a highly Cu^{2+}-selective fluorescent probe derived from rhodamine B

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ABSTRACT

A rhodamine B derivative was synthesized and characterized as a highly selective and sensitive probe for Cu^{2+} in ethanol–water solution (2:3, v:v, pH7.4, 50 mM HEPES). A prominent fluorescence enhancement at 575 nm was observed in the presence of Cu^{2+}, accompanied by the change in the absorption spectrum. Under the optimal conditions, a good linear range of 0.5–1.5 μM with a detection limit of 1.6×10^{-7} M were obtained. Furthermore, confocal laser scanning microscopy experiments have proven that this probe is cell-permeable and can respond to changes in intracellular Cu^{2+} in living cells.

1. Introduction

Fluorescence spectroscopy offers a powerful tool for sensing and imaging trace amounts of species by virtue of its simplicity, high sensitivity and selectivity, and instantaneous response [1–3]. In particular, selective heavy metal ions (HTM) recognition by fluorescence probes has attracted increasing interest in biological and environmental chemistry. Among the HTM, Cu^{2+} plays an important role in living systems and has an extremely ecotoxicological impact on the human health result from its catalytic cofactor for a variety of metalloenzymes, including superoxide dismutase, cytochrome *c* oxidase and tyrosinase [4]. However, Cu^{2+} exhibits toxicity under overloading conditions in that it causes neurodegenerative diseases, probably by its involvement in the production of reactive oxygen species [4]. Owing to the biological significance of Cu^{2+}, a considerable effort has been devoted to the development of the efficient methods to detect Cu^{2+}, and many studies focus on the design of fluorescent probes for the analysis of Cu^{2+} have been reported [5–12]. Whereas most of the reported Cu^{2+} fluorescent probes show "on-off" signal upon the binding of Cu^{2+} due to its paramagnetic nature [5–8], which is not as sensitive as a fluorescence enhancement response [9–12]. Therefore, the development of highly sensitive and selective "off–on" fluorescent probes for Cu^{2+} is still significant.

Based on our previous research [9–14], it is necessary to choose an efficient fluorophore in the design of fluorescent probes. Rhodamine derivative is one of the most useful fluorophores for the construction of artificial fluorescent probes owing to its excellent photophysical properties [15]. In the light of the equilibrium between the spirolactam (non-fluorescence) and the ring-opened amide (fluorescence) of rhodamine derivatives, rhodamine-based probes are ideal modes for in vitro detection and in vivo imaging [16–21]. In addition, the receptor should be preliminarily considered because it is responsible for the selectivity and binding efficiency of the whole probes. According to the Soft-Hard Acid-Base theory, the probes attached the recognition moiety with N and O atoms could show good affinity to Cu^{2+}. Keeping this in mind, a new probe **L** was designed and synthesized as a novel cell membrance-permeable, Cu^{2+}-selective probe in aqueous media and living cells. (Scheme 1).

2. Experimental

2.1. Reagents and instruments

All chemicals were used are of analytical grade or of the highest purity available. Rhodamine B and Hydrazine hydrate (100%) were purchased from Sigma–Aldrich. Glyoxal and other reagents were

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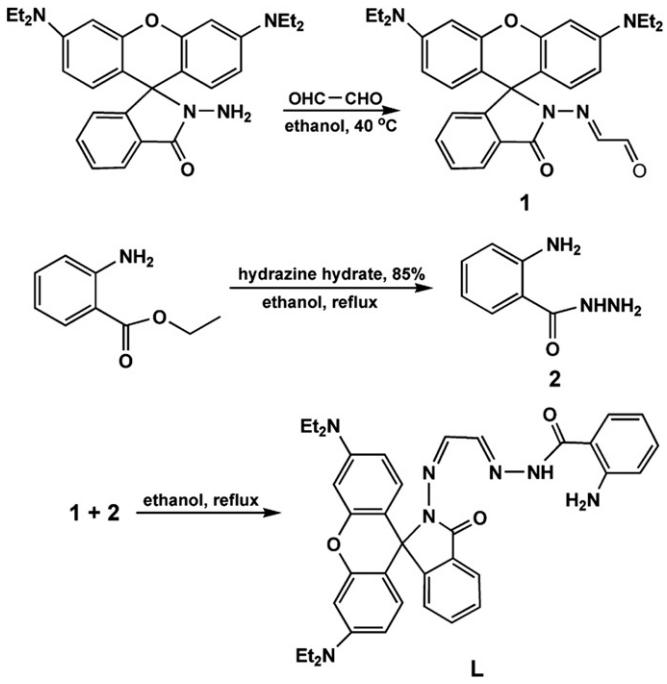


Scheme 1. The synthesis route of probe **L**.

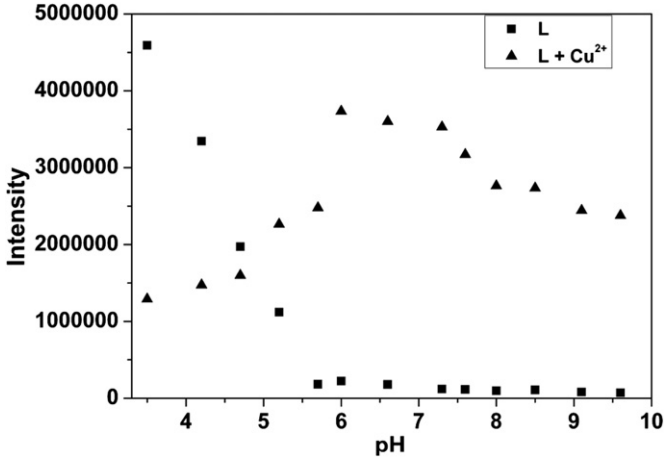


Fig. 1. Influences of pH on the fluorescence spetra of **L** (2 μM) (■) and **L** (2 μM) plus Cu^{2+} (50 μM) (▲) in ethanol–water solution (2:3, v:v). The pH was modulated by adding 1 M HCl or 1 M NaOH in HEPES buffers.

obtained from Shanghai Reagent Company. All solutions were prepared with double–distilled water.

Melting points were determined using a Shanghai Melting points WRS–1B apparatus. NMR spectra were measured with a Brucker WM–500 spectrometer, using TMS as an internal standard. The pH measurements were carried out on a PHS-3C meter. Mass spectra were performed on a Thermo TSQ Quantum Mass Spectrometer. Fluorescence emission spectra were conducted on a HORIBA Fluoromax-4 spectrofluometer. UV–Vis spectra were obtained on a Beckman DU–800 spectrophotometer. Fluorescence imaging was performed by confocal fluorescence microscopy on an Olympus FluoView Fv1000 laser scanning microscope.

2.2. Synthetic procedure

Compounds **1** was synthesized as reported method [21].

The synthesis of compound **2**: Compound **2** was synthesized as reported procedure with some modification [22]. Under N_2 atmosphere, ethyl 2-aminobenzoate (1.0 mmol, 0.165 g) and hydrazine hydrate (12.0 mmol, 0.6 mL) were mixed in 30 mL ethanol. The mixture was refluxed for 6 h. After the reaction was finished, the solvent was removed under reduced pressure, and then 50 mL petroleum ether was added to the oily residue, and the precipitate so produced was filtered and used directly. Yields: 87.2%. M.p.: 152.0–153.1 °C. MS: *m/z* 152.09 $[M + H]^+$.

The synthesis of fluorescent probe **L**: Compound **1** (0.496 g, 1.0 mmol) and compound **2** (0.181 g, 1.2 mmol) were mixed in 30 mL ethanol and refluxed for 4 h. After cooling to room temperature, the precipitate so obtained was washed with water and ethanol, and then dried in vacuum. The **L** was obtained by recrystallization with ethanol as pale yellow solid. Yields: 85.6%. ^1H NMR (d_6-DMSO, δ ppm): 11.64 (s, 1H), 7.99 (d, 1H, $J = 8.10$), 7.42 (d, 1H, $J = 7.45$), 7.82 (d, 1H, $J = 8.20$), 7.60 (t, 1H, $J = 7.47$), 7.54 (t, 1H, $J = 7.26$), 7.43 (d, 1H, $J = 7.85$), 7.18 (t, 1H, $J = 7.67$), 7.45 (d, 1H, $J = 7.55$), 6.02 (d, 1H, $J = 8.05$), 6.53 (t, 1H, $J = 7.87$), 6.45 (s, 2H), 6.41 (s, 2H), 6.36 (d, 2H, $J = 9.00$), 6.33 (b, 2H), 3.32 (m, 8H, $J = 6.95$), 1.09 (t, 12H, $J = 6.97$). ^{13}C NMR (d_6-DMSO, δ ppm): 165.74, 164.71 (C=O), 162.76, 153.51, 152.64, 152.54, 152.34, 150.62, 149.13, 148.60, 146.02, 144.08, 134.91, 133.07, 132.84, 130.10, 129.27, 128.80, 128.58, 128.16, 127.76, 127.50, 124.11 (ArC), 123.97, 123.73 (C=N), 116.86, 115.08, 113.25, 108.67, 108.28, 105.95, 104.83, 97.97, 97.92 (ArC), 65.23, 44.12, 12.89. MS (ESI) *m/z*: 630.4 $[M + H]^+$.

2.3. General spectroscopic methods

Metal ions, anions and fluorescent probe **L** were dissolved in deionized water and DMSO to obtain 1.0 mM stock solutions,

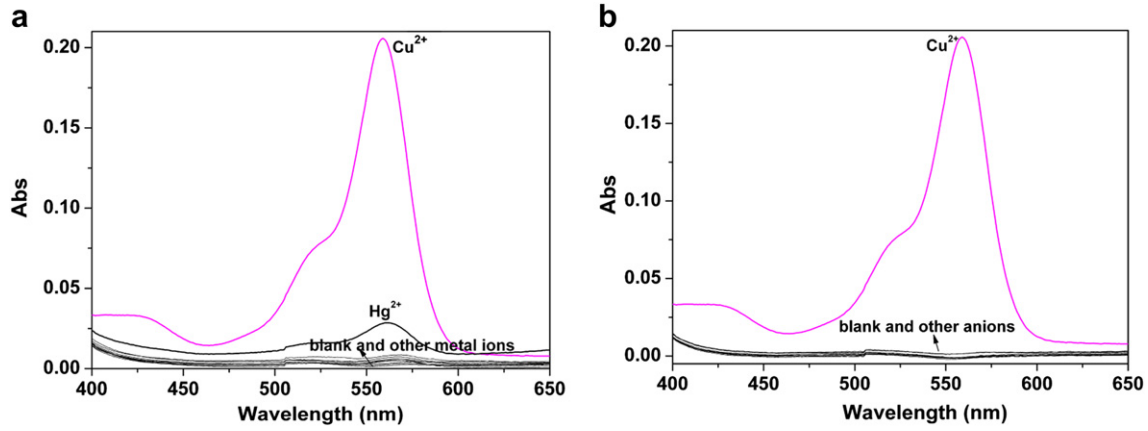


Fig. 2. (a) UV–vis spectra of **L** (2 μM) with different metal ions (50 μM) in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4). (b) UV–vis spectra of **L** (2 μM) with different anions (50 μM) in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4).

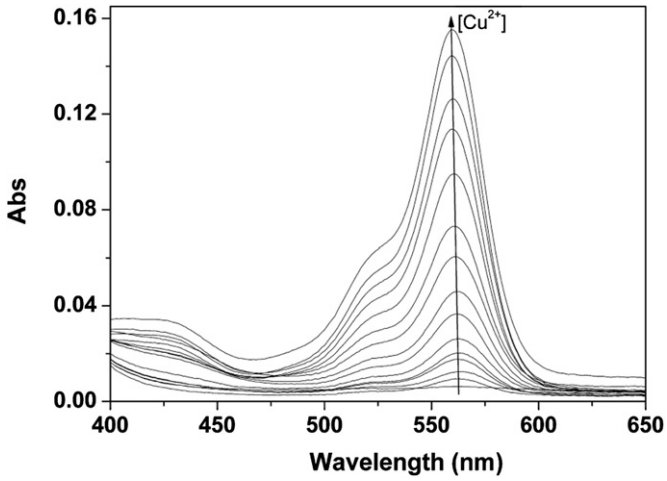


Fig. 3. Absorbance spectra of **L** (2 μM) in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4) in the presence of different amounts of Cu^{2+}.

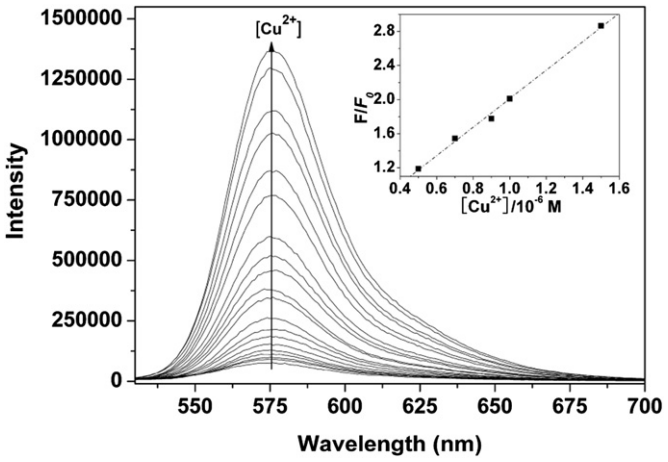


Fig. 5. Fluorescence response of **L** (2 μM) with various concentrations of Cu^{2+} in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4). Inset: the fluorescence at 575 nm of **L** (2 μM) as a function of Cu^{2+} concentrations (0.5–1.5 μM).

respectively. Before spectroscopic measurements, the solution was freshly prepared by diluting the high concentration stock solution to the corresponding solution. For all measurements, excitation and emission slit widths were 4 nm and 4 nm, respectively, excitation wavelength was 520 nm.

2.4. Cell incubation

RAW cells plated on coverslips were washed with phosphate-buffered saline (PBS), followed by incubating with 10 μM of $CuCl_2$ (in PBS) for 30 min at 37 °C, and then washed with PBS three times. After incubating with 20 μM of **L** for 30 min at 37 °C, the cells were washed with PBS three times again.

2.5. Cytotoxicity assay

The in vitro cytotoxicity was measured by using the methyl thiazolyl tetrazolium (MTT) assay in RAW cells. Cells were seeded into 96-well cell culture plate at 4000/well, cultured at 37 °C and 5% CO_2 for 24 h, and then different concentrations of probe **L** (0, 0.1, 1, 10 μM) were added to the wells. The cells were then incubated for 24 h at 37 °C under 5% CO_2. Subsequently, 20 μL MTT (5 mg/mL) was added to each well and incubated for an additional 4 h at 37 °C

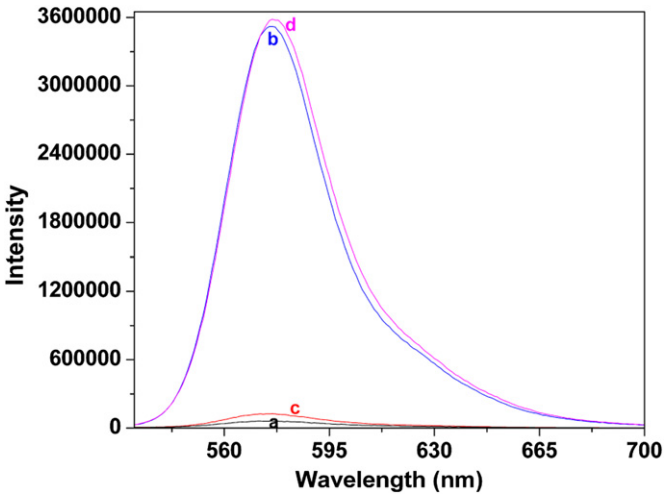


Fig. 6. Fluorescence response in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4): (a) **L** (2 μM); (b) **L** (2 μM) with Cu^{2+} (50 μM); (c) **L** (2 μM) with Cu^{2+} (50 μM) and then addition of EDTA (100 μM); (d) **L** (2 μM) with Cu^{2+} (50 μM) and EDTA (100 μM) and then addition of 100 μM Cu^{2+}.

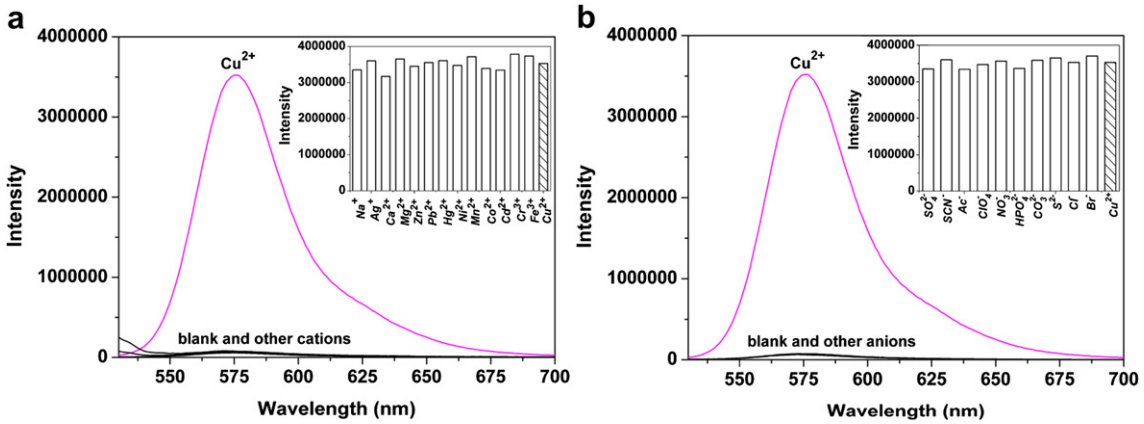
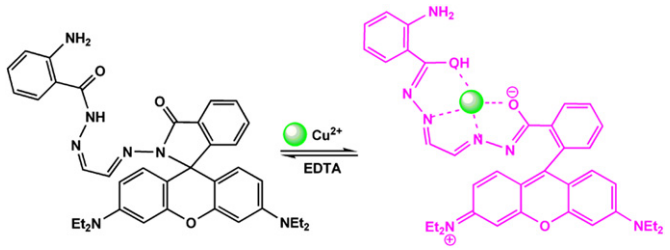


Fig. 4. (a) Fluorescence spectra of **L** (2 μM) with different metal ions (50 μM) in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4). Inset: Fluorescence response of **L** (2 μM) to 50 μM of Cu^{2+} and to the mixture of 250 μM individual other metal ions with 50 μM of Cu^{2+}; (b) Fluorescence spectra of **L** (2 μM) with different anions (50 μM) in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4). Inset: Fluorescence response of **L** (2 μM) to 50 μM of Cu^{2+} and to the mixture of 250 μM individual anions with 50 μM of Cu^{2+}.



Scheme 2. Proposed binding mode of probe **L** toward Cu^{2+}.

under 5% CO_2. Cells were lysed in triple liquid (10% SDS, 0.012 M HCl, 5% isopropanol), and the amount of MTT formazan was qualified by determining the absorbance at 570 nm using a microplate reader (Tecan, Austria).

The following formula was used to calculate the inhibition of cell growth: Cell viability (%) = (mean of Abs. value of treatment group/mean Abs. value of control) • 100%.

3. Results and discussion

3.1. pH investigation

For practical application, the spectra response of **L** in the absence and presence of Cu^{2+} in different pH values were firstly evaluated (Fig. 1). Under acidic conditions (pH < 5.0), ring opening of the rhodamine occurred as a result of protonation. In the pH 5.0–9.0, no obvious characteristic emission of rhodamine could be observed. However, the addition of Cu^{2+} led to the fluorescence enhancement over a comparatively wide pH range of 5.0–9.0, which is attributed to a Cu^{2+}-induced opening of the rhodamine ring. Consequently, **L** may allow Cu^{2+} detection in a wide pH range.

3.2. Uv–vis spectral response of *L*

To validate the selectivity of **L** in pratice, the UV/vis spectrum of **L** to various metal ions and anions are illustrated in Fig. 2a and Fig. 2b, respectively. Upon binding of Cu^{2+}, the absorption spectrum shows the typical rhodamine absorption band at 556 nm, accompanied by a clear color change from colorless to pink. Other metal ions, such as Na^{+}, Ag^{+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{3+}, and anions including SO_4^{2-}, SCN^{-}, Ac^{-}, ClO_4^{-}, NO_3^{-}, HPO_4^{2-}, CO_3^{2-}, S^{2-}, Cl^{-} and Br^{-} did not show any significant color and spectral change under identical conditions, only Hg^{2+} elicited a slight change of absorbance.

Furthermore, absorption titrations of **L** (2 μM) in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4) was conducted (Fig. 3). Upon the gradual addition of Cu^{2+} up to 5 equiv., a new absorption band centered at 556 nm appeared with increasing intensity evidently, clearly indicating the ring-opening process of rhodamine B unit in **L**.

3.3. Fluorescence spectral response of *L*

Fig. 4 shows the fluorescence spectra (ex = 520 nm) of **L** (2 μM) measured in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4) with the addition of respective metal ions and anions. As expected, the addition of Cu^{2+} to the solution of **L** resulted in

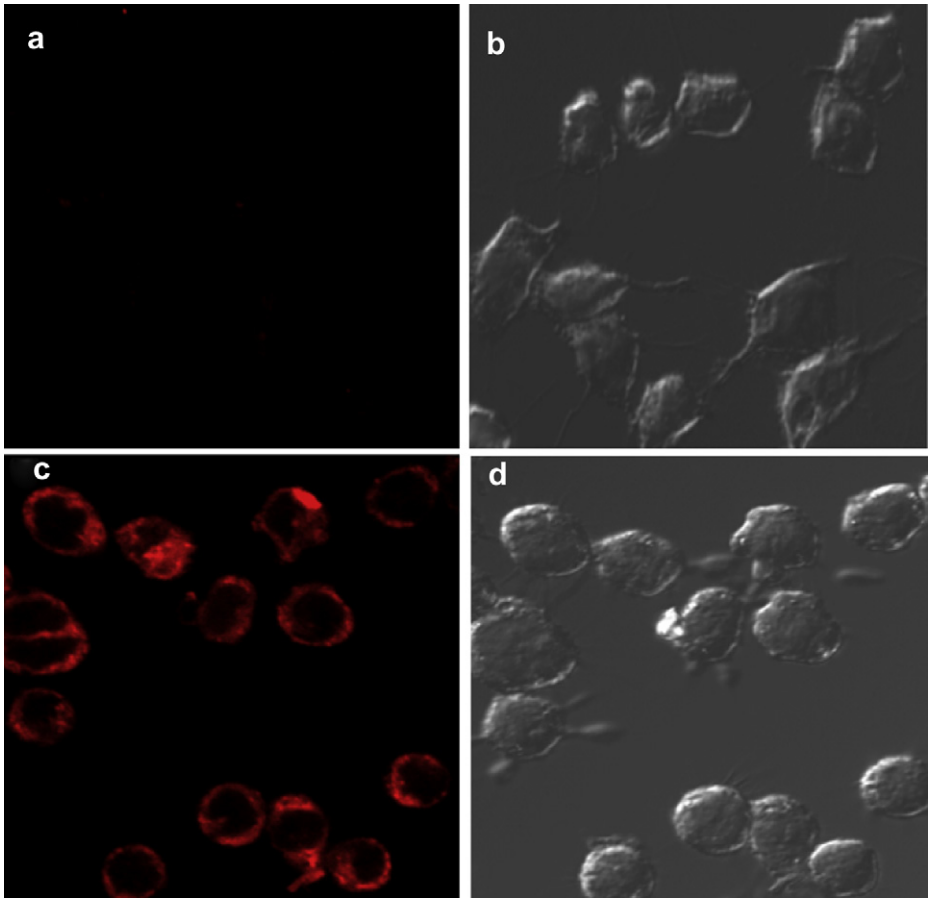


Fig. 7. Confocal fluorescence images in RAW cells. (a) Cells incubated with 20 μM L in PBS buffer for 30 min; (b) Brightfield image of cells shown in panel (a); (c) Cells incubated with 10 μM Cu^{2+} for 30 min, washed three times, and then further incubated with 20 μM L for 30 min (ex = 559 nm); (d) Brightfield image of cells shown in panel (c).

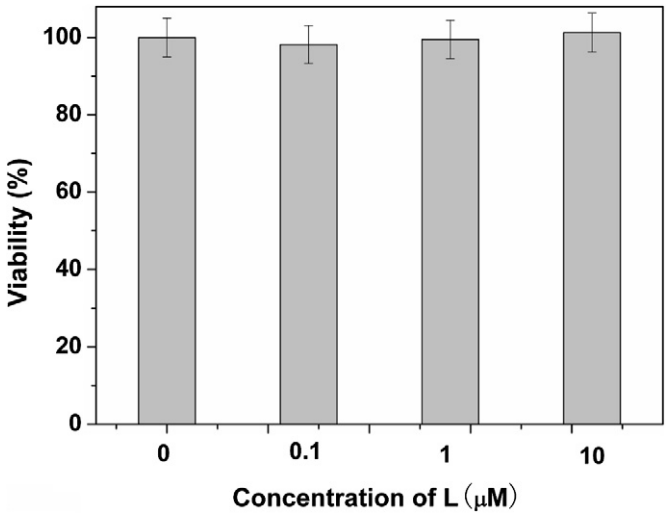


Fig. 8. Cell viability values (%) estimated by MTT proliferation test versus incubation concentrations of **L**. RAW cells were cultured in the presence of 0–10 μM L at 37 °C.

remarkably enhanced fluorescence intensity. Upon interaction with other metal ions and anions, a much weaker response is given compared to Cu^{2+} at the same concentration (50 μM). Moreover, the enhancement of the fluorescence intensity depending on the addition of Cu^{2+} was not suppressed by subsequent addition of other metal ions and anions (Fig. 4 inset). These results indicated that **L** could selectively recognize Cu^{2+} in the presence of miscellaneous competitive metal ions and anions in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4).

To further investigate the interaction of Cu^{2+} and **L**, a fluorescence titration experiment was carried out, as shown in Fig. 5. A linear increase of fluorescence intensity could be observed with increasing Cu^{2+} concentration over a wide range with a detection limit of 1.6×10^{-7} M based on $3 \times \delta_{blank}/k$ (where δ_{blank} is the standard deviation of the blank solution and k is the slope of the calibration plot), suggestingthat the fluorescent probe **L** could sensitively detect environmentally relevant levels of Cu^{2+}.

3.4. The proposed reaction mechanism

To investigate the probable complexation of **L** with Cu^{2+}, the method of continuous variations (Job's plot) is obtained from the **L**-Cu^{2+} system in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4), which clearly suggested the formation of 1:1 stoichiometry between **L** and Cu^{2+} (Supporting Information, Fig. S1). Moreover, the 1:1 stoichiometry mode is also supported by the presence of a peak at m/z 693 corresponding to $[\mathbf{L} + Cu^{2+} - H^+]^+$ in the ESI-MS spectrum of the components of the mixture of **L** and 1 equiv. Cu^{2+} in ethanol (Supporting Information, Fig. S2). The association contant for **L**-Cu^{2+} complex was further estimated to be 9.1×10^4 M^{-1} on the basis of the nonlinear filtting of the fluorescence titration curve assuming a 1:1 stoichiometry by the Benesi-Hildebrand method [17] (Supporting Information, Fig. S3).

To understand the stability of the complex formed, we have analyzed the chemical reversibility behavior of the binding of **L** and Cu^{2+} in the ethanol–water solution. As a consequence, upon addition of 100 μM EDTA to the mixture of **L** (2 μM) and Cu^{2+} (50 μM) in ethanol–water solution (2:3, v:v, 50 mM HEPES, pH7.4), the color changed from pink to almost colorless, and more than 90% fluorescence intensity of the system was quenched (Fig. 6). Then the Cu^{2+} was added to the system, the signals were almost completely recovered, and the colorless solution turned to pink. These findings indicated that **L** is a reversible fluorescent probe for Cu^{2+}. Thus, according to the obtained results, it is very likely due to the metal ion–induced ring opening of rhodamine spirolactam, rather than other possible reactions, and the proposed binding mode for the probe toward Cu^{2+} is illustrated in Scheme 2.

3.5. Preliminary analytical application

In view of its favorable spectroscopic properties, the ability of **L** to detect intracellular Cu^{2+} was also examined in this study. The

Table 1
Performances comparison of various off-on probes for Cu^{2+} derived form rhodamine.

Linear range, μM	LOD, nM	Testing media	Applications	Reproducibility	Ref.
0.05–0.9	3	Water–methanol (8:2, v:v, pH6.0, 20 mM HEPES)	NA[a]	Reversible	[9]
0.1–1.0	85	Water–ethanol (4:6, v:v, pH7.0 50 mM HEPES)	MCF-7 cells	Reversible	[10]
0.05–0.9	7	Water–methanol (2:8, v:v, pH7.0, 20 mM HEPES)	HeLa cells	Reversible	[11]
0.05–4.5	18	Water–ethanol (9:1, v:v, pH7.0, 50 mM HEPES)	HeLa cells	Reversible	[12]
NA	NA	CH_3CN-HEPES (4:6, v:v, pH7.4, 20 mM)	NA	NA	[16]
NA	20	Water-CH_3CN (2:8, v:v, pH7.0, buffered with HEPES)	PC3 cells	Reversible	[18]
NA	NA	CH_3CN-HEPES (5:5, v:v, pH7.4, 20 mM)	NA	NA	[23]
0.001–0.01	NA	Water-CH_3CN (9:1, v:v, pH7.0, 10 mM Tris)	NA	NA	[24]
NA	200	CH_3CN-HEPES (2:8, v:v, pH7.0, 10 mM)	NA	Reversible	[25]
NA	NA	Water-CH_3CN (1:1, v:v)	EJ cells	NA	[26]
NA	NA	Methanol-HEPES (1:1, v:v, pH7.0)	NA	NA	[27]
1–14	10	Water-CH_3CN (1:1, v:v, pH7.1, 10 mM Tris)	Waster water	Reversible	[28]
0–0.005	2	Water-CH_3CN (1:1, v:v, pH7.2, 10 mM Tris)	EJ cells	NA	[29]
10–300	3850	DMSO-Tris (1:9, v:v, pH7.0)	Water sample	Reversible	[30]
2.0–10	280	Water-CH_3CN (3:2, v:v, pH7.0, 10 mM HEPES)	NA	Reversible	[31]
NA	NA	Water-CH_3CN (1:1, v:v)	EJ cells	NA	[32]
NA	NA	Water-CH_3CN (1:1, v:v)	E coli cells	NA	[33]
NA	NA	Water-DMSO (6:4, v:v)	NA	NA	[34]
NA	NA	CH_3CN-Tris (3:1, v:v, pH7.0, 10 mM)	NA	Reversible	[35]
5–55	490	Water-DMF (99:1, v:v, pH5.7)	NA	NA	[36]
NA	6470	Acetate-buffer/DMF (3:3, v:v, pH3.6)	NA	Reversible	[37]
0.25–25	48	Water–ethanol (4:1, v:v, pH8.5, Tris)	NA	Reversible	[38]
0.1–3.0	34	Ethanol-HEPES (1:1, v:v, pH7.2, 20 mM)	HeLa cells	NA	[39]
NA	NA	Water-DMSO (1:10, v:v, pH7.8, 2 mM Tris)	NA	NA	[40]
0.5–1.5	160	Water–ethanol (3:2, v:v, pH7.4, 50 mM HEPES)	RAW cells	Reversible	This work

[a] NA: Not available.

fluorescence images of RAW cells were recorded before and after addition of Cu^{2+} (Fig. 7). The cells were supplemented with only **L** in the growth medium for 30 min at 37 °C, which led to very weak fluorescence as determined by laser scanning confocal microscopy (ex = 559 nm) (Fig. 7(c)). In contrast, the cells were incubated with 10 μM Cu^{2+} in the growth medium for 30 min at 37 °C, and then loaded with **L** under the same conditions, whereupon a bright fluorescence was detected (Fig. 7(a)). These results suggested that fluorescent probe **L** can penetrate the cell membrane and might used for detecting Cu^{2+} in living cells.

To evaluate cytotoxicity of the fluorescent probe, **L** was taken as an example to perform an MTT assay on RAW cells with dye concentrations from 0 μM to 10 μM. The cellular viability estimated was ca. 98% in 48 h after treatment with 10 μM of **L** (Fig. 8), exhibiting low toxicity to cultured cells.

3.6. Method performance comparison

The performance of the proposed probe **L** was compared with some reported probes based on rhodamine B structural motif for Cu^{2+} determination, as shown in Table 1. All the probes present good selectivity for Cu^{2+} with signal enhancement [9–12,16,23–40], and a few of probes possess wide quantitation span [29,38], even down to nM LOD [9,11,29]. But some of them need more rigorous testing media [9,36,38], and the reproductivity [16,23,24,26,27,29,32–34,36,39,40] as well as the applicability in living cells [9,16,23–25,27,31,34–38,40], are not investigated. Thus, there are still numerous challenges and opportunities remaining for development of new probes and practical applications in biological systems. Our proposed probe **L** based on rhodamine is easy to prepare and presents a number of attractive analytical features such as good selectivity, high sensitivity and wide applicability. It can be used for rapid analysis of ultra-trace level Cu^{2+} in living cells with satisfactory results.

4. Conclusion

In summary, we have described an "off-on" type of Cu^{2+} fluorescent probe **L** with an excellent selectivity over other metal ions and anions. Moreover, it has been demonstrated that the fluorescent probe **L** can be used to detect Cu^{2+} in living cells. We expect that this fluorescent probe would help to promote the studies of Cu^{2+} in biological systems.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.dyepig.2012.07.016.

References

[1] Duke RW, Veale EB, Pfeffer FM, Kruger PE, Gunnlaugsson T. Colorimetric and fluorescent anion sensors: an overview of recent developments in the use of 1, 8-naphthalimide-based chemosensors. Chem Soc Rev 2010;39:3936–53.
[2] Kim HN, Guo ZQ, Zhu WH, Yoon JY, Tian H. Recent progress on polymer-based fluorescent and colorimetric chemosensors. Chem Soc Rev 2011;40:79–93.
[3] Zhang JF, Zhou Y, Yoon J, Kim JS. Recent progress in fluorescent and colorimetric chemosensors for detection of precious metal ions (silver, gold and platinum ions). Chem Soc Rev 2011;40:3416–29.
[4] Lovstad RA. A kinetic study on the distribution of Cu(II)-ions between albumin and transferring. BioMetals 2004;17:111–3.
[5] Shao N, Zhang Y, Cheung SM, Yang RH, Chan WH, Mo T, et al. Copper ion-selective fluorescent sensor based on the inner filter effect using a spiropyran derivative. Anal Chem 2005;77:7294–303.
[6] Kim SH, Kim JS, Park SM, Chang SK. Hg^{2+}-selective off-on and Cu^{2+}-selective on-off type fluoroionophore based upon cyclam. Org Lett 2006;8:371–4.
[7] Luo Y, Li Y, Lv BQ, Zhou ZD, Xiao D, Choi MMF. A new luminol derivative as a fluorescent probe for trace analysis of copper(II). Microchim Acta 2009;164:411–7.
[8] Lin W, Yuan L, Tan W, Feng J, Long L. Construction of fluorescent probes via protection/deprotection of functional groups: a ratiometric fluorescent probe for Cu^{2+}. Chem Eur J 2009;15:1030–5.
[9] Yu CW, Zhang J, Wang R, Chen LX. Highly sensitive and selective colorimetric and off-on fluorescent probe for Cu^{2+} based on rhodamine derivative. Org Biomol Chem 2010;8:5277–9.
[10] Zhang J, Yu CW, Qian SY, Lu G, Chen JL. A selective fluorescent chemosensor with 1, 2, 4-triazole as subunit for Cu(II) and its application in imaging Cu(II) in living cells. Dyes Pigm 2012;92:1370–5.
[11] Yu CW, Zhang J, Li JH, Liu P, Wei PH, Chen LX. Fluorescent probe for copper(II) ion based on a rhodamine spirolactame derivative, and its application to fluorescent imaging in living cells. Microchim Acta 2011;174:247–55.
[12] Yu CW, Chen LX, Zhang J, Li JH, Liu P, Wang WH, et al. "Off-On" based fluorescent fluorescent probe for Cu^{2+} in aqueous media and living cells. Talanta 2011;85:1627–33.
[13] Zhang J, Yu CW, Lu G, Fu QY, Li N, Ji YX. Ag^{+}-selective "off-on" probe based on napthalimide derivative. New J Chem 2012;36:819–22.
[14] Yu CW, Zhang J, Chen LX. Silver(I) ion-only sensing in an aqueous media based on "off-on" fluorescent probe. Anal Methods 2012;4:342–4.
[15] Kim H, Lee M, Kim H, Kim J, Yoon J. A new trend in rhodamine-based fluorescent probes: application of spirolactam ring-opening to sensing ions. Chem Soc Rev 2008;37:1465–72.
[16] Zhou Y, Wang F, Kim Y, Kim SJ, Yoon J. Cu^{2+}-selective ratiometric and "off-on" sensor based on the rhodamine derivative bearing pyrene group. Org Lett 2009;11:4442–5.
[17] Zhao Y, Zhang XB, Han ZX, Qiao L, Li CY, Jian LX, et al. Highly sensitive and selective colorimetric and off-on fluorescent chemosensor for Cu^{2+} in aqueous solution and living cells. Anal Chem 2009;81:7022–30.
[18] Kumar M, Kumar N, Bhalla V, Sharma PR, Kaur T. Highly selective fluorescence turn-on chemodosimeter based on rhodamine for nanomolar detection of copper ions. Org Lett 2012;14:406–9.
[19] Lee MH, Giap TV, Kim SH, Lee YH, Kang C, Kim JS. A novel strategy to selectively detect Fe(III) in aqueous media driven by hydrolysis of a rhodamine 6G schiff base. Chem Commun 2010;46:1407–9.
[20] Huang JH, Xu YF, Qian XH. A rhodamine-based Hg^{2+} sensor with high selectivity and sensitivity in aqueous solution: a NS_2-containing receptor. J Org Chem 2009;74:2167–70.
[21] Du JJ, Fan JL, Peng XJ, Sun PP, Wang JY, Li HL, et al. A new fluorescent chemodosimeter for Hg^{2+}: selectivity, sensitivity, and resistance to Cys and GSH. Org Lett 2010;12:476–9.
[22] Gup R, Giziroglu E. Metal complexes and solvent extraction properties of isonitrosoacetophenone 2-aminobenzoylhydrazone. Spectrochim Acta A 2006;65:719–26.
[23] Zhang JF, Zhou Y, Yoon J, Kim Y, Kim SJ, Kim JS. Naphthalimide modified rhodamine derivative: ratiometric and selective fluorescent sensor for Cu^{2+} based on two different approaches. Org Lett 2010;12:3852–5.
[24] Liu YL, Sun Y, Du J, Lv X, Zhao Y, Chen ML, et al. Highly sensitive and selective turn-on fluorescent and chromogenic probe for Cu^{2+} and ClO^{-} based on a *N*-picolinyl rhodamine B-hydrazide derivative. Org Biomol Chem 2011;9:432–7.
[25] Dong M, Ma TH, Zhang AJ, Dong YM, Wang YW, Peng Y. A series of highly sensitive and selective fluorescent and colorimetric "off-on" chemosensors for Cu(II) based on rhodamine derivatives. Dyes Pigm 2010;87:164–72.
[26] Huang L, Wang X, Xie GQ, Xi PX, Li ZP, Xu M, et al. A new rhodamine-based chemosensor for Cu^{2+} and the study of its behaviour in living cells. Dalton Trans 2010;39:7894–6.
[27] Huo FJ, Su J, Sun YQ, Yin CX, Tong HB, Nie ZX. A rhodamine-based dual chemosensor for the visual detection of copper and the ratiometric fluorescent detection of vanadium. Dyes Pigm 2010;86:50–5.
[28] Hua ZQ, Wang XM, Feng YC, Ding L, Lu HY. Sulfonyl rhodamine hydrazide: a sensitive and selective chromogenic and fluorescent chemodosimeter for copper ion in aqueous media. Dyes Pigm 2011;88:257–61.
[29] Huang L, Hou FP, Xi PX, Bai DC, Xu M, Li ZP, et al. A rhodamine-based "turn-on" fluorescent chemodosimeter for Cu^{2+} and its application in living cell imaging. J Inorg Biochem 2011;105:800–5.
[30] Xu ZH, Zhang LK, Guo R, Xiang TC, Wu CZ, Zheng Z, et al. A highly sensitive and selective colorimetric and off–on fluorescent chemosensor for Cu^{2+} based on rhodamine B derivative. Sens Actuators B 2011;156:546–52.
[31] Tang LJ, Li FF, Liu MH, Nandhakumar R. A new rhodamine B derivative as a colorimetric chemosensor for recognition of copper(II) ion. Bull Korean Chem Soc 2010;31:3212–6.

[32] Huang L, Chen FJ, Xi PX, Xie GQ, Li ZP, Shi YJ, et al. A turn-on fluorescent chemosensor for Cu^{2+} in aqueous media and its application to bioimaging. Dyes Pigm 2011;90:265–8.
[33] Chereddy NR, Thennarasu S. Synthesis of a highly selective bis-rhodamine chemosensor for naked-eye detection of Cu^{2+} ions and its application in bio-imaging. Dyes Pigm 2011;91:378–82.
[34] Li TR, Yang ZY, Li Y, Liu ZC, Qi GF, Wang BD. A novel fluorescein derivative as a colorimetric chemosensor for detecting copper(II) ion. Dyes Pigm 2011;88:103–8.
[35] Weerasinghe AJ, Abebe FA, Sinn E. Rhodamine based turn-on dual sensor for Fe^{3+} and Cu^{2+}. Tetrahedron Lett 2011;52:5648–51.
[36] Zhou YM, Zhang JL, Zhou H, Zhang QY, Ma TS, Niu JY. A new rhodamine B-based "off-on" fluorescent chemosensor for Cu^{2+} in aqueous media. J Lumin 2012;132:1837–41.
[37] Ding JH, Yuan LD, Gao L, Chen JW. Fluorescence quenching of a rhodamine derivative: selectivity sensing Cu^{2+} in acidic aqueous media. J Lumin 2012; 132:1987–93.
[38] Zhang LF, Zhao JL, Zeng X, Mu L, Jiang XK, Deng M, et al. Turning with pH: the selectivity of a new rhodamine B derivative chemosensor for Fe^{3+} and Cu^{2+}. Sens Actuators B 2011;160: 662–9.
[39] Liu WY, Li HY, Zhao BX, Miao JY. A new fluorescent and colorimetric probe for Cu^{2+} in living cells. Analyst 2012;137:3466–9.
[40] Xu L, Xu YF, Zhu WP, Zeng BB, Yang CM, Wu B, et al. Versatile trifunctional chemosensor of rhodamine derivative for Zn^{2+}, Cu^{2+} and His/Cys in aqueous solution and living cells. Org Biomol Chem 2011;9: 8284–7.