Highly sensitive and selective chemosensor for Cu^{2+} detection based on a N-propargyl rhodamine 6G-hydrazide derivative

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A novel fluorescence “turn-on” probe for Cu^{2+} detection has been designed based on a Cu^{2+} triggered spirolactam ring-opening reaction. The synthetic probe is a double-responsive fluorescent and colorimetric Cu^{2+} -specific sensor. In aqueous solution, it exhibits high selectivity and excellent sensitivity. With a significant color change visible to the naked eye at the concentration of 3 μM (ca. 0.19 mg/L), about one magnitude lower than the WHO (World Health Organization) recommended level (2.0 mg/L) for Cu^{2+} ions in drinking water, the probe could be used to monitor Cu^{2+} ions in drinking water.

copper, fluorescent chemosensor, chromogenic probe, rhodamine

1 Introduction

The development of highly selective and sensitive methods for detection and visualization of metal ions with biological interest is a very attractive research topic in the chemosensing and molecular imaging fields [1]. Among various transition metal ions, Cu^{2+} is the third most abundant transition metal in the human body [2], and plays a critical role as a catalytic cofactor for a variety of metalloenzymes and transcriptional events [3]. However, under overloading conditions, Cu^{2+} exhibits toxicity which has been reported to associate with Alzheimer's and Wilson's diseases probably by its involvement in the production of reactive oxygen species [4, 5]. In recent years, Cu^{2+} has also been suspected to cause infant liver damage [6]. Accordingly, the World Health Organization (WHO) has set the maximum allowable level of Cu^{2+} in drinking water at 2.0 ppm ($\sim 30 \mu\text{M}$) [7]. Nevertheless, Cu^{2+} contamination and its potential toxic effects on human beings continue to be challenging problems

throughout the world due to the widespread use of Cu^{2+} in agriculture and industry [8]. Therefore, a fast, convenient, and reliable method for Cu^{2+} detection is required, particularly in drinking water monitoring. Sensors based on the ion-induced changes in fluorescence appear to be particularly attractive because of the simplicity, high sensitivity, and low detection limit of the fluorescence [9]. Recently, rhodamine derivatives have been utilized as useful fluorophores due to their excellent spectroscopic properties of large molar extinction coefficient and high fluorescence quantum yield. The fluorescent chemosensors based on the rhodamine derivatives have been used as Pb^{2+} sensor [10], Hg^{2+} sensor [11, 12], Fe^{3+} sensor [13], Cr^{3+} sensor [14], Ag^+ sensor [15] and Cu^{2+} sensor [16–21]. Most of rhodamine derivatives are nonfluorescent and colorless, whereas addition of targeted metal cation leading to ring-opening of the corresponding spirolactam gives rise to strong fluorescence emission and a pink color. Based on the understanding of the sensing mechanism of rhodamine-based molecular sensors, herein, we disclose a design and synthesis of a new rhodamine-based chemosensor **1**, which shows a highly selective and sensitive fluorescence enhancement in re-

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sponse to Cu^{2+} in complex aqueous samples. The improvement in the color response, as well as rapid and accurate recognition of Cu^{2+} with naked eye will make this new approach a very promising alternative for the detection of Cu^{2+} in water media.

2 Experimental

2.1 General experimental

All chemicals were of reagent grade and used without further purification. Ultrapure water with a Millipore Purification System (Milli-Q water) was used throughout the analytical experiments. Fluorescence spectra were obtained using a Hitachi F-7000 Fluorescence Spectrometer equipped with a xenon lamp, 1.0 cm quartz cells, and slits of 1.0/1.0 nm. UV spectra were measured with a Beckman Du-800 Spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on ARX 400 spectrometers for solutions in CDCl_3 or CD_3OD . Chemical shifts are given in ppm downfield from internal Me_4Si . High-resolution mass spectrometry was conducted in a positive mode with ESI-source. Thin layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (V/V) H_2SO_4 in MeOH or in some cases by a UV detector.

2.2 Synthesis of compound 1

Rhodamine 6G hydrazide (**2**) was prepared from Rhodamine 6G according to a literature procedure [22]. The chemicals involved in the synthesis of probe **1** are shown in Scheme 1.

To a solution of Rhodamine 6G hydrazide (**2**) (500 mg, 1.17 mmol) in THF (25 mL) sodium hydride (72 mg, 1.8 mmol) in THF (5 mL) was added drop-by-drop at 0°C and stirred for 30 min. A solution of propargyl bromide (**3**) (160 mg, 1.29 mmol) in THF (5 mL) was then added slowly and the mixture was stirred overnight under reflux. The reaction mixture was quenched with water (50 mL) and extracted with ethyl acetate (3×100 mL). The combined organic layer was dried over anhydrous Na_2SO_4 , concentrated under vacuum, and the residue was purified by flash column chromatography to give **1** a colorless solid (325 mg, 60%). ^1H NMR (400 MHz, CDCl_3), δ (TMS, ppm): 1.32 (t, $J = 7.0$ Hz, 6H), 1.90 (s, 6H), 2.10 (t, $J = 2.4$ Hz, 1H), 3.18–3.24 (m, 6H), 3.51 (s, 2H), 4.61 (s, 1 H), 6.28 (s, 2H), 6.32 (s, 2H),

7.05–7.07 (m, 1H), 7.44–7.47 (m, 2H), 7.93–7.95 (m, 1H). ^{13}C NMR (CDCl_3 , 100 MHz), δ (TMS, ppm): 14.76, 16.69, 38.37, 40.35, 65.48, 72.36, 80.06, 96.78, 105.95, 117.65, 122.90, 123.93, 128.11, 128.28, 129.71, 132.82, 147.41, 151.94, 152.0, 166.59. ESI(+)-HRMS (m/z): $[\text{M} + \text{H}]^+$ calcd. for $\text{C}_{29}\text{H}_{31}\text{N}_4\text{O}_2$: 467.2447, found 467.2442.

2.3 General procedure for Cu^{2+} detection

All the measurements were conducted according to the following procedures. 0.10 mL of probe **1** (1.0 mM) containing 20% of CH_3CN was blended with 0.10 mL of Cu^{2+} solution in HEPES acid buffer (20 mM, pH 5.5) in a 10 mL colorimetric tube. After addition of 20 mM of HEPES acid buffer to 10.00 mL, the mixture was equilibrated for 2 min and then the fluorescence intensity was recorded at $\lambda_{\text{ex}}/\lambda_{\text{em}} = 535/575$ nm alongside a reagent blank. The excitation and emission slits were both set at 1.0 nm.

3 Results and discussion

3.1 Selectivity of probe 1 to Cu^{2+}

Probe **1** is soluble in an aqueous medium containing a co-solvent of CH_3CN (20%, V/V). Although the solution itself is non-fluorescent and colorless, in the presence of Cu^{2+} , a rhodamine-type intermediate with a strong fluorescence signal (Figure 1) and a pink color visible to the naked eye (Figure 2) are produced. This phenomenon is caused by

Figure 1 The variation of fluorescence intensity ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 535/575$ nm) of probe **1** (10 μM) in the presence of 10 μM Cu^{2+} in 20 mM HEPES acid buffer with different contents of co-solvent CH_3CN in aqueous media.

Scheme 1 Synthesis of rhodamine-based chemosensor **1**.

copper chelation with propargyl, which induces ring-opening at C–N bond in spirolactam. The study showed that there was no significant fluorescence signal observed for probe **1** solution alone (Figure 2(a)), while a strong fluorescent peak rapidly appeared in the presence of Cu^{2+} (Figure 2(b)). The maximum excitation wavelength of test solution was at 535 nm, and the maximum emission wavelength at 575 nm. The effects of pH on probe **1** and reaction with Cu^{2+} were evaluated. It was found that varying pH from 5.5 to 11 did not show significant fluorescent change for probe **1** itself, and the relative fluorescent intensity was not strongly pH-dependent over the range of 5 to 6 for the reaction system (Figure 3).

The selectivity of probe **1** for Cu^{2+} against other individual cation ions was investigated by adding probe **1** (10 μM) to the aqueous solution of Cu^{2+} (10 μM) and other metal ions Ag^+ , Ca^{2+} , Cd^{2+} , Co^{2+} , Hg^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , and Zn^{2+} (100 μM), respectively. As shown in Figure 4, only Cu^{2+} induced a significant fluorescence signal, and appeared a pink color from the colorless test solution (probe **1** as blank). The fluorescent intensity leveled off after 200 s (Figure 5). Addition of other cation ions up to a concentration as high as 100 μM , caused no detectable increase of both fluorescent signal and color changes to the naked eye.

3.2 Sensitivity of probe **1**

Figure 6 shows the emission fluorescence spectra of probe **1** vs. Cu^{2+} contents. When the Cu^{2+} concentration increased from 0 to 10 μM while exciting at 535 nm, the emission fluorescence intensity of probe **1** at 575 nm increased more than 28-fold. A satisfactory linear relationship between fluorescence intensity and Cu^{2+} concentration was observed with the correlation coefficient as high as 0.9994, the linear relationship was still 0.997 even when Cu^{2+} concentration ranged from 0 to 1.0 μM . Similarly, as shown in Figure 7, the UV absorbance at 530 nm enhanced from 0.006 to 0.099 when increasing concentration of Cu^{2+} from 0 to 10 μM . A satisfactory linear relationship between UV-vis absorbance and Cu^{2+} concentration was observed with the correlation coefficient as high as 0.9981. Based on the Job's plot analysis, probe **1** reacted with Cu^{2+} in a 1:1 ratio as expected (Figure 8). Similar to many reported rhodamine spirolactam-based fluorescent chemosensors, the fluorescence enhancement response of probe **1** toward Cu^{2+} is most likely the result of the spirolactam ring-opening mechanism (Scheme 2). The probe solution was titrated with Cu^{2+} and then subjected to the mass spectrometry analysis. The mass spectrum of the solution displayed only one main peak at

Figure 2 Fluorescence spectra ($\lambda_{\text{ex}} = 535$ nm) and naked-eye color of probe **1** (10 μM), recorded in 20 mM HEPES acid buffer (pH 5.5) containing 20% (V/V) CH_3CN before (a) and after (b) addition of Cu^{2+} (10 μM) at room temperature.

Figure 3 (a) The variation of fluorescence intensity ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 535/575$ nm) of probe **1** (10 μM) in 20 mM HEPES acid buffer (pH 5.5) containing 20% CH_3CN over a pH range of 4 to 11 at room temperature; (b) the variation of fluorescence intensity ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 535/575$ nm) of probe **1** in the presence of 10 μM Cu^{2+} in 20 mM HEPES acid buffer with different pH values from 4.5 to 7.

Figure 4 Fluorescence spectra ($\lambda_{\text{ex}} = 535 \text{ nm}$) of probe **1** ($10 \mu\text{M}$) in 20 mM HEPES acid buffer ($\text{pH } 5.5$) containing $20\% \text{ (V/V)}$ CH_3CN with $10 \mu\text{M}$ Cu^{2+} and $100 \mu\text{M}$ other metal ions (Ag^+ , Ca^{2+} , Cd^{2+} , Co^{2+} , Hg^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , Zn^{2+}).

m/z 465.2 (Figure 9), which was characterized to be compound $[4+\text{H}]^+$ (m/z_{calc} 465.2).

Scheme 2 The proposed interaction mechanism of probe **1** with Cu^{2+} .

Figure 6 (a) Fluorescence titration spectra ($\lambda_{\text{ex}} = 535 \text{ nm}$) of probe **1** ($10 \mu\text{M}$) with Cu^{2+} from 0 to $10 \mu\text{M}$ in 20 mM HEPES acid buffer ($\text{pH } 5.5$) containing $20\% \text{ (V/V)}$ CH_3CN ; (b) the plot of fluorescence intensity change of probe **1** ($10 \mu\text{M}$) against varied concentration of Cu^{2+} from 0 to $10 \mu\text{M}$ at $\lambda_{\text{ex}}/\lambda_{\text{em}} = 535/575 \text{ nm}$. Inset: The plot of fluorescence intensity change of probe **1** ($10 \mu\text{M}$) against varied concentration of Cu^{2+} from 0 to $1.0 \mu\text{M}$.

Figure 5 The change of fluorescence intensity ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 535/575 \text{ nm}$) of probe **1** ($10 \mu\text{M}$) with time in the presence of $10 \mu\text{M}$ Cu^{2+} in 20 mM HEPES acid buffer ($\text{pH } 5.5$) containing $20\% \text{ CH}_3\text{CN}$ at room temperature.

Furthermore, the detection limit of Cu^{2+} in HEPES acid buffer ($\text{pH } 5.5$) with the synthetic probe **1** was determined using both S/N test method and serial dilution method. It was found that the detection limit of fluorescent method

Figure 7 UV-vis absorption titration spectra of probe **1** (10 μM) with Cu^{2+} from 0 to 10 μM in 20 mM HEPES acid buffer (pH 5.5) containing 20% (V/V) CH_3CN . Inset: The plot of UV-vis absorbance change of probe **1** (10 μM) against varied concentration of Cu^{2+} from 0 to 10 μM at $\lambda = 530$ nm.

Figure 8 Job plot: the relationship between probe **1** + Cu^{2+} and relative fluorescence intensity. The total concentration of probe **1** and Cu^{2+} was kept at a constant 10 μM . Excitation was provided at 535 nm and the emission intensity was measured at 575 nm. Spectra were acquired in 20 mM HEPES acid buffer (pH 5.5). The data are consistent with a 1:1 (Cu^{2+} : probe **1**) complex.

Figure 9 The mass spectra of produced compound **4**.

Figure 10 "Naked eye" color changes of probe **1** (1 equiv. to Cu^{2+}) in 20 mM HEPES acid buffer (pH 5.5) containing 20% CH_3CN under natural light upon addition of different concentration of Cu^{2+} at room temperature. (a) Cu^{2+} ranged from 0 to 9 μM ; (b) Cu^{2+} ranged from 0 to 50 μM .

was as low as 0.65 μM (41.6 $\mu\text{g/L}$), and the relative standard deviation of eleven runs was 7.3%. The detection limit (41.6 $\mu\text{g/L}$) of probe **1** to Cu^{2+} is 48 times lower than the recommended water quality standard of Cu^{2+} (2.0 mg/L) for drinking water by WHO [7], EU [23], and Australia [24]. The present result implies that probe **1** can be simply, rapidly, and satisfactorily used to detect Cu^{2+} concentration. Apparently, under naked-eye conditions, the pink color of

the solution is of correlation with the concentrations of probe **1** and Cu^{2+} . Figure 10 shows the color variations of probe **1** solution in terms of Cu^{2+} concentration. It can be seen that the color changes could be exclusively confirmed with naked-eye when the Cu^{2+} concentration increased to 3 μM (ca. 0.19 mg/L), ten times lower than the recommended standard value for Cu^{2+} in drinking water by WHO, EU and Australia. This observation suggested that probe **1** could be

Figure 11 The prepared color chart according to the solution color with different concentrations of Cu^{2+} ion.

used conveniently to screen the Cu^{2+} concentration in the aqueous sample with naked eye. Based on the optical variation of probe **1**, a color chart was prepared according to the correlation of color change and Cu^{2+} concentration. As shown in Figure 11, the Cu^{2+} concentration in water sample could be identified through comparing the color in test vial and chart with naked eye.

4 Conclusion

In summary, we have designed, synthesized, and investigated the properties of a new fluorescent probe **1** for Cu^{2+} detection based on a Cu^{2+} triggered ring-opening reaction of spiro lactam in aqueous medium. Probe **1** is a dual-responsive fluorescent and colorimetric Cu^{2+} -specific sensor in aqueous media. Fluorescent probe **1** exhibits an excellent Cu^{2+} ions selectivity over other cations, as well as a satisfactory detection limit at $41.6 \mu\text{g/L}$. More importantly, the probe displays a significant naked-eye color change with increasing Cu^{2+} concentration. The formed pink color can be easily recognized and distinguished by the naked eye even at $3.0 \mu\text{M}$ (ca. 0.19 mg/L) of Cu^{2+} . Since this level is about one magnitude lower than the WHO recommended maximum drinking water value of 2.0 mg/L for Cu^{2+} . The probe could be used to monitor Cu^{2+} in drinking water. A color chart showing the colors and corresponding Cu^{2+} concentrations was prepared to provide a potential method for visible detection of Cu^{2+} ions. We anticipate that the rapid and accurate naked-eye identification of Cu^{2+} , as well as the satisfactory detection limit, would make this approach a very promising choice for Cu^{2+} detection in water media. Moreover, the molecular design presented here may contribute to the development of more efficient and more useful chemosensors based on the rhodamine platform.

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