A New Rhodamine B Derivative As a Colorimetric Chemosensor for Recognition of Copper(II) Ion

Lijun Tang,* Fangfang Li, Minghui Liu, and Raju Nandhakumar†,*

College of Chemistry and Chemical Engineering, Liaoning Key Laboratory for the Synthesis and Application of Functional Compounds, Bohai University, Jinzhou 121013, P. R. China. *E-mail: lijuntang@tom.com
†Department of Chemistry, Karunya University, Karunya Nagar, Coimbatore - 641114, TamilNadu, India †E-mail: rajunandhakumar@yahoo.com

Received August 28, 2010, Accepted September 7, 2010

A new rhodamine-based sensor was designed and synthesized by incorporating rhodamine B and benzimidazole moieties. Sensor 1 exhibits high selectivity and sensitivity to Cu²⁺ in CH₃CN-water solution (HEPES buffer, pH = 7.0) with an obvious color change from colorless to pink. Other metal ions such as Hg²⁺, Ag⁺, Pb²⁺, Sr²⁺, Ba²⁺, Cd²⁺, Ni²⁺, Co²⁺, Fe²⁺, Mn²⁺, Cu²⁺, Zn²⁺, Ce³⁺, Mg²⁺, K⁺ and Na⁺ had no such color change and have no significant influence on Cu²⁺ recognition process. The interaction of Cu²⁺ and sensor 1 was proven to adopt a 1:1 binding stoichiometry and the recognition process is reversible.

Key Words: Colorimetric, Chemosensor, Rhodamine B, Copper recognition

Introduction

Copper being an essential trace element for plants and animals, is the third most abundant transition metal following zinc and iron in human bodies.¹ It plays a crucial role in many fundamental physiological processes in organisms. For instance, it serves as a catalytic cofactor for a variety of metalloenzymes, including superoxide dismutase, cytochrome c oxidase and tyrosinase.² Whereas, under overloading conditions, copper exhibits toxicity associated with neurodegenerative diseases such as Alzheimer’s disease and prion diseases.³ In addition, it has been suspected to cause infant liver damage in recent years.⁴ Due to its janus-faced properties of copper ion in organisms, considerable attention has been devoted to the design of efficient and selective colorimetric chemosensors for Cu²⁺ because of its simplicity, quick and nondestructive advantages of the absorption method.⁵

Rhodamines are an important class of fluorogenic and chromogenic probes, which are the ideal platforms for development of colorimetric chemosensors for specific heavy and transition metal ions. They have excellent spectroscopic properties such as long-wavelength emission, high fluorescence quantum yield, and large molar extinction coefficient. Recently, utilization of rhodamine spirolactam ring-opening process for the detection of metal ions have been well documented.⁶ The spirolactam form is basically colorless, while the metal induced ring-opened amide form gives rise to strong absorption within the visible range,⁷ this feature provides a vital pathway for colorimetric detection of metal ions.

Herein, we designed a new benzimidazole containing rhodamine B derivative, chemosensor 1, for colorimetric recognition of Cu²⁺. It is well-known that benzimidazole moiety has been widely applied as copper binding ligands due to its high affinity toward Cu²⁺.⁸ Therefore, we speculated that by incorporating the 2-aminobenzimidazole moiety into a rhodamine-based probe would (1) increase the affinity and selectivity to Cu²⁺ in competitive aqueous media, (2) realize real-time detection of Cu²⁺ through colorimetric response, and (3) recognize Cu²⁺ reversibly.

Experimental Section

General methods and materials. ¹H NMR spectra and ¹³C NMR spectra were obtained on a Varian INOVA-400 MHz and Bruker AV-300 MHz Spectrometer, respectively. Chemical shifts were expressed in ppm and tetramethylsilane (TMS) was used as internal standard. High-resolution mass spectrometry (HRMS) was carried out on a UPLC/Q Tof mass spectrometer. UV spectra were measured on a SP-1900 spectrophotometer at room temperature. Chemical reagents for synthesis obtained commercially were used without further purification.

Synthesis of sensor 1. To a stirred mixture of rhodamine B (2.0 g, 4.52 mmol) and 2-aminobenzimidazole (0.72 g, 5.43 mmol) in 30 mL of anhydrous acetonitrile was added a few drops of phosphorus oxychloride at room temperature, and heated to reflux for 4 h. After cooled to room temperature, the resulting solution was poured into 200 mL of cold water, and extracted with dichloromethane. The organic layer was then washed with aqueous NaOH solution and dried over anhydrous Na₂SO₄. Then the solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography with ethyl acetate-methanol (10:1, v/v) as eluent to give sensor 1. Yield: 1.56 g (62%), white solid, mp. 156 - 157 °C. ¹H NMR
A Rhodamine B Derivative As Chemosensor for Copper

(400 MHz, CDCl₃) δ 11.16 (s, 1H), 8.04 (d, 1H, J = 8.0 Hz), 7.60-7.52 (m, 2H), 7.44 (s, 1H), 7.30 (s, 1H), 7.21 (d, 1H, J = 8.0 Hz), 7.07-7.03 (m, 2H), 6.48 (s, 2H), 6.41 (d, 2H, J = 8.0 Hz), 6.14 (d, 2H, J = 8.0 Hz), 3.30 (q, 8H, J = 8.0 Hz), 1.14 (t, 12H, J = 8.0 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 153.8, 148.6, 134.3, 131.4, 128.5, 127.7, 124.7, 123.3, 121.3, 119.1, 109.9, 107.2, 106.3, 97.7, 44.2, 12.6; HRMS-ESI [M+H]+ calcd. m/z (558.2869), found m/z (558.2885).

Results and Discussion

Chemosensor 1 was readily prepared by a one-step condensation of rhodamine B and 2-aminobenzimidazole in anhydrous acetonitrile in the presence of a catalytic amount of phosphorus oxychloride. The structure of 1 was confirmed by NMR and high resolution mass spectroscopy, and the results are in good agreement with the structure presented.

Our preliminary absorption spectra studies showed that sensor 1 has a good selectivity to Cu²⁺ in CH₃CN-water (2:3, v/v, HEPES 10 mM, pH = 7.0) solution. Solution of sensor 1 is colorless and has no absorption in the visible range. Upon addition of Cu²⁺, the color changes to pink and a new absorption band centered at 557 nm was formed. We then investigated the time evolution of the responses of 1 (10 µM) in the presence of 20 equiv of Cu²⁺ in CH₃CN-water (2:3, v/v, HEPES 10 mM, pH = 7.0) because a short time response is necessary for a chemosensor to monitor metal ions in aqueous media. As shown in Figure 1, upon addition of 20 equiv of Cu²⁺ to 1 solution, the absorption intensity at 557 nm of the tested solution reaches maximum in 2 min and does not change with more reaction time, which indicates that the interaction of 1 and Cu²⁺ was completed in 2 min. Hence, 1 is a sensitive Cu²⁺ sensor and could be applied in real-time detection of Cu²⁺ in environmental analysis. Therefore all the absorption spectral data in this work are recorded at 2 min after the addition of ionic species.

The sensing property of 1 toward Cu²⁺ was then investigated by incremental addition of Cu²⁺ to 1 solution. As shown in Figure 2, upon addition of Cu²⁺ (0 to 8.0 equiv), the new absorption band centered at 557 nm increased gradually, which corresponds to the ring-opening process of the spirolactam form of 1. Linear fitting of the titration profiles using Benesi-Hildebrand plot based on a 1:1 binding mode results in a good linearity (correlation coefficient is over 0.99) (Fig. 3), which strongly support the 1:1 binding stoichiometry of 1 and Cu²⁺, and the binding constant is found to be 4.27 × 10⁵ M⁻¹.¹⁰

The 1:1 binding stoichiometry of Cu²⁺ and 1 was further proved by Job’s plot according to the continuous variations with a total concentration of [Cu²⁺] + [1] as 5.0 × 10⁻⁵ M. (Fig. 4). The absorbance exhibited a maximum when the molar fraction of Cu²⁺ was 0.5, which also demonstrates the 1:1 binding stoichiometry is adopted between 1 and Cu²⁺.

To further validate the selectivity of 1 to Cu²⁺ over other metal ions, the absorption response of 1 (10 µM) toward various metal ions was conducted by UV-vis absorption in CH₃CN-water (2:3, v/v, HEPES 10 mM, pH = 7.0) solution and the results are shown in Figure 5. The free sensor 1 remained colorless and did not exhibit apparent absorption above 450 nm in the aforementioned buffer solution. Upon addition of 8.0 equiv of Cu²⁺ into the colorless solution of 1, a new strong absorption band responds to the ring-opening process of the spirolactam form of 1.
Figure 4. Job’ plot monitored at 557 nm, the total concentration of \([\text{Cu}^{2+}] + [1]\) was \(5.0 \times 10^{-5}\) M.

Figure 5. The absorption spectra of 1 (1.0 \(\times\) \(10^{-5}\) M) upon addition of various metal ions.

Figure 6. The absorbance of 1 (1.0 \(\times\) \(10^{-5}\) M) at 557 nm to various metal ions. The grey bars represent the absorption of 1 in the presence of 8.0 equiv of miscellaneous metal ions, the red bars represent the absorption of the above solution upon addition of 8.0 equiv of Cu\(^{2+}\). 1. Ni\(^{2+}\), 2. Hg\(^{2+}\), 3. Ba\(^{2+}\), 4. Ag\(^{+}\), 5. Mg\(^{2+}\), 6. Fe\(^{3+}\), 7. K\(^{+}\), 8. Ce\(^{3+}\), 9. Mn\(^{2+}\), 10. Pb\(^{2+}\), 11. Na\(^{+}\), 12. Sr\(^{2+}\), 13. Co\(^{2+}\), 14. Zn\(^{2+}\), 15. Cd\(^{2+}\), 16. Cr\(^{3+}\), 17. Fe\(^{3+}\), 18. Cu\(^{2+}\).

Figure 7. Absorption intensity of solution 1 versus the concentration of Cu\(^{2+}\) in the low concentration range (2.0 \(\times\) \(10^{-6}\) to 1.0 \(\times\) \(10^{-5}\) M).

In addition, to check its practical utility, the colorimetric detection limit of 1 for Cu\(^{2+}\) was evaluated. Under the present conditions, a good linear relationship between the absorption intensity and the Cu\(^{2+}\) concentration could be obtained in the 2.0 \(\times\) \(10^{-6}\) to 1.0 \(\times\) \(10^{-5}\) M range (R = 0.9969) (Fig. 7). The detection limit is then calculated to be 2.8 \(\times\) \(10^{-7}\) M with the equation:

\[
\text{detection limit} = 3S/\rho,
\]

where \(S\) is the standard deviation of blank measurements, \(\rho\) is the slope between intensity versus sample concentration. This result indicates that our colorimetric Cu\(^{2+}\) probe 1 is sensitive enough to monitor Cu\(^{2+}\) concentration in water.

Furthermore, the effect of pH on the absorbance of 1 in CH\(_3\)CN-water (2.3, v/v, 10 mM HEPES, pH = 7.0) was determined. The acid-base titration experiments showed that sensor 1 has a very weak absorption at pH 6 and has no absorption at pH greater than 7 (Fig. 8). This result revealed that 1 is suitable for detection of Cu\(^{2+}\) at near neutral pH conditions.
salt (EDTA-Na₂) was selected as the titration reagent due to its high affinity to Cu²⁺. Accordingly, a HEPES buffered solution was added, this result revealed the response of the binding of sensor 1 with Cu²⁺ in the CH₃CN-water buffer (1.0 × 10⁻⁵ M) and Cu²⁺ (8.0 × 10⁻⁵ M) and Cu²⁺ was proved to be reversible and Cu²⁺ is reversible rather than a cation catalyzed reaction. After coordination to Cu²⁺, sensor 1 only exhibited the characteristic color change, and did not generate the strong fluorescence emission of rhodamine type. This can be attributed to the quenching effect by paramagnetic Cu²⁺. Similar phenomena was also observed in other experiments.¹²

The plausible binding mode of 1 with Cu²⁺ that led to the absorbance changes is proposed in Scheme 2. According to the proposed mechanism of some rhodamine-based chemosensor for metal ions,⁶-¹³ it can be supposed that the oxygen atom of the carbonyl group as well as a nitrogen atom on the benzimidazole moiety can cooperatively participate in the binding with Cu²⁺ and induce the ring-opening of the spirolactam.

**Conclusion**

In summary, we developed a new and simple rhodamine-based colorimetric chemosensor 1, which displays high selectivity and sensitivity toward Cu²⁺ and shows no significant response to other evaluated metal ions. The Cu²⁺ recognition process of 1 is not significantly influenced by other coexisting metal ions. The interaction of 1 and Cu²⁺ is proved to be reversible with a 1:1 binding stoichiometry, and the detection limit is found to be 2.8 × 10⁻⁷ M.

**Acknowledgments.** This work was supported by the foundation of educational department of liaoning province (No: 2008-T002).

**References**

mun. 2007, 3069-3070.