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A rhodamine-benzothiazole conjugated sensor for colorimetric, ratiometric and sequential recognition of copper(II) and sulfide in aqueous media

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Abstract: A new rhodamine-benzothiazole conjugated colorimetric sensor 1 that exhibits sequential recognition to Cu²⁺ and S²⁻ in CH₃CN/HEPES buffer (v/v = 1:1, HEPES 10 mM, pH = 7.0) solution has been developed. Sensor 1 displays highly selective and sensitive recognition to Cu²⁺ with a ratiometric behavior, and the resultant 1-Cu²⁺ complex can act as a highly selective S²⁻ sensor via Cu²⁺ displacement approach. The Cu²⁺ and S²⁻ recognition processes are rapid and reversible, and the Cu²⁺ and S²⁻ inputs can result in an INHIBIT logic gate.

Keywords: rhodamine; benzothiazole; copper(II) recognition; sulfide recognition; colorimetric

Introduction

The development of artificial receptors for the sensing and recognition of environmentally and biologically important ionic species, especially transition metal ions, is currently of great interest [1]. Copper, as the third most abundant transition metal in the human body, is vital for both environmental and biological systems [2, 3]. Nevertheless, at high concentrations, copper becomes a toxic and hazardous elements to organisms such as leading to gastrointestinal disturbance and the damage to the liver and kidneys [4, 5].

Also, in recent years, the detection of anions gains more and more concerns. As one of the biologically and environmentally important anions [6], sulfide anions are widely generated as a byproduct in industrial processes, for instance, conversion into sulfur, preparation of sulfuric acid and dyes, cosmetic manufacturing, production of wood pulp, etc [7-9]. Although sulfide has many industrial utilities, exposure to high levels of sulfide can lead to various physiological and biochemical problems including irritation in mucous membranes, unconsciousness, and respiratory paralysis [10]. Therefore, sulfide detection has received immense interest and a number of...
sulfide selective probes based on irreversible sulfide-specific triggered reactions have been reported [11-13]. Nevertheless, most of the organic reactions were time-consuming and required relatively strict conditions, which limited the probes application. Development of chemosensors based on metal ion displacement (especially utilizing a Cu\(^{2+}\) affinity) has been proved to be an effective method to realize real-time and reversible sulfide detection [14-16].

Recently, we reported some simple and selective fluorescent and colorimetric sensors for relay recognition of Cu\(^{2+}\) and S\(^{2-}\) ions [17-19]. However, most of the works depend on fluorescence signal “on-off” switching at the same position, which usually suffer from the interferences of environmental conditions and are prone to hamper their accuracy detection. Utilization of ratiometric chemosensor is an effective method to hurdle this issue due to the built-in correction property of ratiometric sensors [20]. Rhodamine chromophore has been widely employed to construct sensors for naked eye observable sensing of metal ions due to its well-known spirolactam to ring-opened amide equilibrium [21-24]. Incorporation of a chromophore with metal ion binding sites into rhodamine moiety is a good approach to construct ratiometric and colorimetric metal ion sensors.

Herein, we designed and synthesized a new rhodamine-benzothiazole conjugated chemosensor 1, which exhibits a rapid, selective, sensitive and reversible recognition to Cu(II) via ratiometric UV-vis absorption changes in neutral buffered media [25, 26]. Moreover, the on site generated 1-Cu\(^{2+}\) complex displays highly selective and sensitive recognition to S\(^{2-}\). Thus, a colorimetric and ratiometric sequential recognition of Cu\(^{2+}\) and S\(^{2-}\) has been achieved.

**Scheme 1.**

**Experimental**

*Materials and instruments*

Unless otherwise stated, solvents and reagents were purchased as analytical grade and used without further purification. Ethanol was freshly distilled from Magnesium powder and Iodine grain. Doubly distilled water was used for spectral detection. Compounds 2 [27] and 4 [28] were prepared according to literature methods,
respectively. \(^1\)H NMR and \(^{13}\)C NMR spectra were recorded on Agilent 400-MR spectrometer. Low-resolution mass spectroscopy (LRMS) was measured on an Agilent 1100 series LC/MSD mass spectrometer. High-resolution mass spectroscopy (HRMS) was measured on a Bruker micrOTOF-Q mass spectrometer (Bruker Daltonik, Bremen, Germany). UV-vis absorption spectra were measured on a SP-1900 spectrophotometer (Shanghai Spectrum instruments Co., Ltd., China). The pH measurements were made with a PHS-25B meter (Shanghai Dapu instruments Co., Ltd., China).

**General spectroscopic procedures**

Sensor 1 was dissolved in CH\(_3\)CN/HEPES buffer (v/v = 1:1, HEPES 10 mM, pH = 7.0) to give the test solution (10 \(\mu\)M). Titration experiments were carried out in 10-mm quartz cuvettes at 25 °C. Water solution (HEPES 10 mM, pH = 7.0) of metal ions (as chloride or nitrate salts, 10 mM) and anions (as sodium or potassium salts) were used in the spectroscopic study. 1-Cu\(^{2+}\) solution for S\(^2-\) detection was prepared by addition of 7 equiv. of Cu\(^{2+}\) to sensor 1 solution (10 \(\mu\)M) in CH\(_3\)CN/HEPES buffer (v/v = 1:1, HEPES 10 mM, pH = 7.0).

**Synthesis of 3-(benzo[d]thiazol-2-yl)-2-hydroxy-5-methylbenzaldehyde (3)**

Compound 2 (450 mg, mmol) was dissolved in CF\(_3\)COOH (7.0 mL), then hexamethylenetetramine (570 mg, mmol) was added in one portion. The resulting mixture was refluxed and monitored by TLC until the starting material disappeared. After slow addition of 10 mL water, the resulted mixture was refluxed for another 10 min. The precipitates formed was collected by filtration and washed with distilled water. Yield 92%. m.p. 171-172 °C. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 12.72 (s, 1H), 10.32 (s, 1H), 8.19 (d, \(J = 8.0\) Hz, 2H), 8.10 (d, \(J = 7.8\) Hz, 1H), 7.71 (d, \(J = 1.6\) Hz, 1H), 7.58 (t, \(J = 7.6\) Hz, 1H), 7.49 (t, \(J = 7.6\) Hz, 1H), 2.38 (s, 3H). \(^{13}\)C NMR (100 MHz, DMSO-d\(_6\)) \(\delta\) 193.0, 167.4, 159.2, 153.2, 137.5, 135.3, 131.5, 129.1, 128.0, 125.4, 124.5, 121.3, 21.9. LRMS (API-ES+). Calcd for C\(_{43}\)H\(_{42}\)N\(_5\)O\(_3\)S [M+H]\(^+\): m/z 270.1, Found: m/z 270.0.

**Synthesis of** 2-((3-(benzo[d]thiazol-2-yl)-2-hydroxy-5-methylbenzylidene)amino)-3',6'-bis(diethyla
Compounds 3 (100 mg, 0.37 mmol) and 4 (170 mg, 0.37 mmol) were dissolved in 30 mL of absolute ethanol and the mixture was heated to reflux for 6 hrs. After the reaction completed, the solvent was rotary evaporated, the residue was purified by column chromatography with ethyl acetate-petroleum ether (1:3, v/v) as eluent affording 180 mg of 1 as pale yellow solid. Yield: 68%. m.p. 252-253 °C. 1H NMR (400 MHz, DMSO-d$_6$) δ 12.35 (s, 1H), 8.82 (s, 1H), 8.04 (d, $J$ = 8.0 Hz, 1H), 7.99-7.92 (m, 3H), 7.64-7.55 (m, 3H), 7.49 (t, $J$ = 7.6 Hz, 1H), 7.40 (t, $J$ = 7.6 Hz, 1H), 7.24 (s, 1H), 7.09 (d, $J$ = 7.6 Hz, 1H), 6.45-6.38 (m, 3H), 6.31 (d, $J$ = 8.4 Hz, 2H), 3.25 (dd, $J$ = 6.8 Hz, 8H), 2.20 (s, 3H), 1.01 (t, $J$ = 6.8 Hz, 12H). 13C NMR (100 MHz, DMSO-d$_6$) δ 164.2, 154.1, 153.0, 151.6, 148.8, 134.8, 133.0, 132.0, 131.1, 129.5, 129.3, 129.1, 128.4, 128.1, 127.1, 125.8, 124.3, 123.7, 122.6, 122.3, 120.2, 119.4, 109.1, 98.3, 98.2, 65.5, 44.4, 20.3, 12.7. HRMS (ESI+). Calcd for C$_{43}$H$_{42}$N$_5$O$_3$S [M+H]$^+$: m/z 708.3008, Found: m/z 708.2952.

Result and discussion

Colorimetric recognition of 1 to Cu$^{2+}$

Sensor 1 was synthesized by the method depicted in Scheme 1. The rhodamine B spirolactam moiety was selected as potential signaling unit to sense Cu$^{2+}$, which was envisioned to turn-on the visible color when it bound to Cu$^{2+}$ [29]. On the other hand, the benzothiazole moiety introduced to sensor 1 with the hypothesis that Cu$^{2+}$ binding will interrupt the original absorption property of benzothiazole skeleton.

The UV-vis absorption property of probe 1 (10 µM) in CH$_3$CN/HEPES buffer (v/v = 1:1, pH = 7.0) was firstly investigated (Fig. 1). Free sensor 1 showed an absorption band at 368 nm, which is attributed to the benzothiazole moiety. The absence of absorption band in the visible region indicates that the rhodamine moiety in sensor 1 [30] exists mainly in its spirolactam form [31]. Upon addition 7.0 equiv. of Cu$^{2+}$ to 1 solution, the absorption band at 368 nm greatly decreased, simultaneously, two new absorption bands at 420 nm and 556 nm developed, respectively. As expected, a distinct color change from colorless to pink was also observed, indicative of the Cu$^{2+}$ binding induced rhodamine spirolactam ring opening event [32]. Addition of Fe$^{2+}$ (7.0
equiv.) elicited absorption intensity decrease at 368 nm and appearance of new absorption bands at 446 nm and 556 nm, however, the band intensity at 556 nm is rather small. Introducing Co\textsuperscript{2+} only can promote one new absorption band at 466 nm. However, addition of other interested metal ions including Hg\textsuperscript{2+}, Ag\textsuperscript{+}, Pb\textsuperscript{2+}, Sr\textsuperscript{2+}, Ba\textsuperscript{2+}, Cd\textsuperscript{2+}, Ni\textsuperscript{2+}, Mn\textsuperscript{2+}, Fe\textsuperscript{3+}, Zn\textsuperscript{2+}, Al\textsuperscript{3+}, Cr\textsuperscript{3+}, Mg\textsuperscript{2+}, K\textsuperscript{+}, Ca\textsuperscript{2+}, and Na\textsuperscript{+} did not produce any significant absorption spectrum changes of sensor 1. In addition, the colorimetric response of 1 to Cu\textsuperscript{2+} could finish within 40 s (Fig. S1). This result suggests that sensor 1 could serve as a rapid, highly selective and “naked-eye” detectable colorimetric sensor for Cu\textsuperscript{2+} in neutral buffered media.

**Figure 1.**

To get an insight into the sensing properties of 1 to Cu\textsuperscript{2+}, titration experiments were then performed. As depicted in figure 2, on incremental addition of Cu\textsuperscript{2+} to 1 solution, the initial absorption band decreased gradually, and the newly generated bands at 420 nm and 556 nm increased accordingly. The absorbance became constant when 7.0 equiv. of Cu\textsuperscript{2+} was employed. It is noteworthy that the Cu\textsuperscript{2+} induced absorption changes showed a ratiometric behavior between concentration ranges from 10 to 50 µM when the two absorption ratio (\(A_{556\text{ nm}}/A_{368\text{ nm}}\)) was monitored (Fig 2, inset). From the titration profile, the association constant (\(K_a\)) of 1 and Cu\textsuperscript{2+} was estimated to be 1.01x10\textsuperscript{6} M\textsuperscript{-1} \cite{33, 34} by non-linear least squares fitting of the titration data based on a 1:1 binding equation (\(R^2 = 0.99813\)) (Fig. S2), and the detection limit of sensor 1 to Cu\textsuperscript{2+} was estimated to be 6.89x10\textsuperscript{-6} M (Fig. S3) through fitting the normalized absorption intensity versus Log[Cu\textsuperscript{2+}] \cite{35}. Moreover, the formation of 1-Cu\textsuperscript{2+} complex with 1:1 stoichiometry was strongly supported by the mass spectrometry analyses, in which the prominent peak appeared at \(m/z\) 769.2141 is assignable to \([1+Cu^{2+}\text{-H}^+]^{+}\) species (Fig. S4).

**Figure 2.**

To explore the ability of sensor 1 for Cu\textsuperscript{2+} detection, competition experiments were taken into account (Fig. 3). The absorbance changes of sensor 1 to Cu\textsuperscript{2+} in the presence of other competitive metal ions were examined. Except for Cu\textsuperscript{2+}, other potential competitive metal ions promoted almost no changes of sensor 1 solution.
These results indicate that coexistence of other metal ion promoted no obvious influence on Cu$^{2+}$ detection. This implies that sensor 1 can be used in environmental and biological systems for Cu$^{2+}$ detection even in the presence of other competitive metal ions.

**Figure 3.**

Effects of pH on absorbance changes (at 556 nm) of sensor 1 with and without 7.0 equiv. of Cu$^{2+}$ were also investigated (Fig. 4) to evaluate the practical applicability. The results show that sensor 1 behaves almost no absorbance in a wide pH range from 4 to 13. On addition of Cu$^{2+}$ to sensor 1 solution at different pH conditions, dramatic absorbance enhancement was observed from pH 4 to 10. Such a wide pH range response of 1 to Cu$^{2+}$ make sensor 1 has a potential practical applicability for Cu$^{2+}$ detection in physiological and environmental systems.

**Figure 4.**

To further understand the binding mode of 1 with Cu$^{2+}$, a several of quantum chemical calculations with the DFT/B3LYP method have been carried out to get the possible configurations of 1-Cu$^{2+}$. The 6-31G basis set was adopted for H, N, and O atoms, for Cu atom, the LANL2DZ effective core potential was used. The calculated energy-minimized structure reveals that the Cu$^{2+}$ ion chelates to 1 very well through three coordination sites including lactim O, imine N, and phenolic O atoms (Fig. S5).

*Colorimetric recognition of S$^{2-}$ by 1-Cu$^{2+}$ complex*

Application of metal-chromaphore complex as anion selective sensor based on metal ion displacement approach has been proved to an effective method for anion sensing. To assess whether our 1-Cu$^{2+}$ complex could be used as an anion selective chemosignaling system, the response of 1-Cu$^{2+}$ complex toward physiologically and environmentally important anions were then evaluated. As shown in Fig. 5, upon addition of 7.0 equiv. of S$^{2-}$ to 1-Cu$^{2+}$ (consist 10 µM of 1 and 70 µM of Cu$^{2+}$) solution, the 1-Cu$^{2+}$ absorption bands restored to the original absorption band of 1, concomitantly, the solution color changed from pink to colorless. This result reveals that the bound Cu$^{2+}$ in 1-Cu$^{2+}$ complex was completely sequestered by S$^{2-}$, which induces releasing of free 1. Whereas, addition of other anions such as other
physiological and environmental important anions, including F-, Cl-, Br-, I-, SCN-, CN-, S2O32-, SO32-, HSO3-, PO43-, H2PO4-, HPO42-, NO2-, NO3-, AcO-, ClO4-, SO42-, P2O74-, HSO4-, CO32-, and HCO3- to 1-Cu2+ solution do not produce any discernible absorption spectra changes. Thus, 1-Cu2+ complex can serve as a highly selective sensor for S2-. From the abovementioned results, the proposed mechanism for S2- sensing of 1-Cu2+ can be illustrated in Scheme 2.

**Figure 5.**

**Scheme 2.**

For better understanding the S2- sensing property, the ensemble solution was titrated with sulfide anions (Fig. 6). The UV-vis spectral pattern of the titration experiment was similar but in reserve direction to the titration curve obtained with Cu2+. This fact is evidence that sensor 1 is regained from complex in presence of S2-. When 7 equiv. of S2- was added, the absorbance came to be constant. The detection limit of S2- calculated to be 8.13×10^{-6} M (Fig. S6). In addition, the effect of pH on S2- detection of 1-Cu2+ solution was also conducted. In the presence of 7 equiv. of S2-, the absorption intensities (at 556 nm) of the tested 1-Cu2+ solutions were greatly decreased in a pH range from 4 to 10, especially at near neutral pH conditions (Fig. S7). This result indicates that the in situ generated 1-Cu2+ complex is practically applicable for S2- detection in a wide pH range.

**Figure 6.**

To investigated whether the 1-Cu2+ complex could still retain its sensing response to S2- under the potential competition of other relevant anions, the probe 1-Cu2+ was treated with S2- (7.0 equiv.) in the presence of various test anions (7.0 equiv.) in CH3CN/H2O (1:1, v/v, HEPES 10 mM). As displayed in Figure 7, all the relevant anions tested have virtually no influence on the UV-vis spectral detection of S2-. Thus, the ensemble is useful for selectively sensing S2- in physiological solution.

**Figure 7.**

The spectral responses of 1 to Cu2+ and the resultant complex 1-Cu2+ to S2- proved that both Cu2+ and S2- recognition processes are reversible. Alternating addition of equal amount of Cu2+ and S2- to 1 solution led to alternate increasing and decreasing
in absorbance at 556 nm (Fig. 8, left). This ‘ON–OFF–ON’ switching processes could be repeated several times, suggesting the potential applicability of 1 for sequential detection of Cu$^{2+}$ and S$^{2-}$ in some real samples.

Due to the sequential and reversible recognition behavior of 1 to Cu$^{2+}$ and S$^{2-}$, an integrated and sequential logic circuit can be constructed [36, 37]. Depending on the two chemical inputs, namely, input 1 (Cu$^{2+}$) and input 2 (S$^{2-}$), sensor 1 can switch between high and low UV absorbance states. Input 1 elicits strong absorption at 556 nm, represent as output ‘1’. On the contrary, input 2 causes no strong absorption signal, represent as output ‘0’. Hence, monitoring the absorbance at 556 nm, upon addition of Cu$^{2+}$, S$^{2-}$, and their reacting dose mixture results in an INHIBIT logic gate (Fig. 8, right).

**Figure 8.**

**Conclusions**

In summary, a new rhodamine-benzothiazole conjugated colorimetric sensor 1 has been designed and synthesized. Sensor 1 displays highly selective, sensitive and ratiometric recognition to Cu$^{2+}$ in CH$_3$CN/HEPES buffer (v/v = 1:1, pH = 7.0) solution. The in situ generated 1-Cu$^{2+}$ complex behaves as a S$^{2-}$ sensor with high sensitivity and selectivity through metal ion displacement approach. Both the Cu$^{2+}$ and S$^{2-}$ recognition processes are rapid and reversible, and the Cu$^{2+}$ and S$^{2-}$ inputs exhibit an INHIBIT logic gate property. Thus, a highly selective colorimetric sensor for sequential recognition of Cu$^{2+}$ and S$^{2-}$ has been achieved. These features make sensor 1 has the potential applicability in environmental systems for Cu$^{2+}$ and S$^{2-}$ detection.

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**Appendix A. Supplementary material**

Supplementary data associated with this article can be found in the online version.
References

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Scheme 1. Synthesis of sensor 1.

Scheme 2. Proposed sequential sensing mechanisms of 1 for Cu$^{2+}$ and S$^{2-}$.
Figure 1. The absorption spectra of 1 (10 μM) in CH$_3$CN/H$_2$O (1:1, v/v, HEPES 10 mM, pH = 7.0) solution upon addition of 7.0 equiv of various metal ions. Metal ions including Hg$^{2+}$, Ag$^+$, Pb$^{2+}$, Sr$^{2+}$, Ba$^{2+}$, Cd$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Fe$^{3+}$, Zn$^{2+}$, Al$^{3+}$, Cr$^{3+}$, Mg$^{2+}$, K$^+$, Ca$^{2+}$, and Na$^+$. Inset: the color changes of 1 before and after Cu$^{2+}$ addition.

Figure 2. Changes in absorption spectra of 1 (10 µM) in CH$_3$CN/H$_2$O (1:1, v/v, HEPES 10 mM, pH = 7.0) solution upon addition of different amount Cu$^{2+}$ (0 to 7.0 equiv.). Inset: Absorbance ratio ($A_{556\text{ nm}}/A_{368\text{ nm}}$) changes on addition of Cu$^{2+}$ (0 to 7.0 equiv.) in aqueous solution (HEPES 10 mM, pH = 7.0).
Figure 3. The absorption responses of 1 solution (10 μM) at 556 nm to various metal ions. The black bars represent the absorption of 1 in the presence of 7.0 equiv. of miscellaneous metal ions, the red bars represent the absorption of the above solution upon further addition of 7.0 equiv. of Cu$^{2+}$.

Figure 4. The absorption response (556 nm) of 1 and 1-Cu$^{2+}$ solutions at different pH conditions in CH$_3$CN/H$_2$O (1:1, v/v, HEPES 10 mM).
Figure 5. The absorption spectra changes of 1-Cu$^{2+}$ (10 μM) in CH$_3$CN/H$_2$O (1:1, v/v, HEPES 10 mM, pH = 7.0) in the presence of 7 equiv. of different anions. Other anions including F-, Cl-, Br-, I-, SCN-, CN-, S$_2$O$_3^{2-}$, SO$_3^{2-}$, HSO$_3^-$, PO$_4^{3-}$, H$_2$PO$_4^-$, HPO$_4^{2-}$, NO$_2^-$, NO$_3^-$, AcO-, ClO$_4^-$, SO$_4^{2-}$, P$_2$O$_7^{4-}$, HS$_2$O$_8^-$, CO$_3^{2-}$, and HCO$_3^-$.  

Figure 6. The absorption spectra changes of 1-Cu$^{2+}$ solution (10 μM) in CH$_3$CN/H$_2$O (1:1, v/v, HEPES 10 mM, pH = 7.0) on incremental addition of S$_2^-$ (0 to 7.0 equiv.).
Figure 7. Changes of absorbance (556 nm) of 1-Cu$^{2+}$ solution (10 μM) in CH$_3$CN/H$_2$O (1:1, v/v, HEPES 10 mM, pH = 7.0). The black bars represent the absorbance spectra of 1-Cu$^{2+}$ solution in the presence of 7.0 equiv. of different anions. The red bars represent the absorbance spectra of 1-Cu$^{2+}$ to S$^{2-}$ solution in the presence of others anions.

Figure 8. Absorbance changes (556 nm) of 1 solution (10 μM) upon alternating addition of Cu$^{2+}$/S$^{2-}$ (left) and the truth table and logic scheme (right).
A rhodamine-benzothiazole conjugated sensor for colorimetric, ratiometric and sequential recognition of copper(II) and sulfide in aqueous media

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Graphical abstract

A new rhodamine-benzothiazole conjugated colorimetric sensor 1 that exhibits sequential recognition to Cu$^{2+}$ and S$^{2-}$ in CH$_3$CN/HEPES buffer (v/v = 1:1, pH = 7.0) solution has been developed.
Highlights
1) A new rhodamine-benzothiazole conjugated colorimetric sensor 1 has been designed and synthesized.
2) Sensor 1 displays sequential recognition to Cu$^{2+}$ and S$^{2-}$ in neutral aqueous solution through naked eye observable color changes.
3) Sensor 1 exhibits ratiometric absorption changes during Cu$^{2+}$ and S$^{2-}$ sequential recognition processes.
4) The Cu$^{2+}$ and S$^{2-}$ recognition are highly selective and sensitive.
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