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PAPER

Solvent controlled sugar–rhodamine fluorescence sensor for Cu²⁺ detection†Xing Ma,^{ab} Zhuowei Tan,^a Guohua Wei,^{*a} Dongbin Wei^{*a} and Yuguo Du^{*ab}

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A “turn-on” fluorescence probe for Cu²⁺ detection has been reported according to a Cu²⁺ triggered spiroactam ring-opening reaction. The probe is a double-responsive fluorescent and colorimetric Cu²⁺-specific sensor in aqueous solution containing 20% of acetonitrile with high selectivity and excellent sensitivity (limit of detection is 12 μg L⁻¹). Furthermore, the significant color changes visible to the naked eye at the concentration of 3 μM (*ca.* 0.20 mg L⁻¹) are about ten times lower than the WHO (World Health Organization) recommended level (2.0 mg L⁻¹) for Cu²⁺ ions in drinking water.

1. Introduction

Copper is the third-most abundant transition metal in the human body,¹ and plays a critical role as a catalytic cofactor for a variety of metalloenzymes and transcriptional events.² However, it was reported that overloaded Cu²⁺ might stimulate the production of reactive oxygen species and thus exhibits toxicity associated with Alzheimer's and Wilson's diseases.^{3,4} In recent years, Cu²⁺ has also been suspected to cause infant liver damage.⁵ Accordingly, the World Health Organization (WHO) has set the maximum allowable level of copper in drinking water at 2.0 ppm ($\sim 30 \mu\text{M}$).⁶ Nevertheless, copper contamination and its potential toxic effects on human beings continue to be challenging problems throughout the world due to the widespread use of Cu²⁺ in agriculture and industry.⁷ Therefore, a fast, convenient, and reliable method for Cu²⁺ detection is required, particularly in drinking water monitoring. Fluorescent chemosensors with high specificity and sensitivity, ease and safety of handling have received considerable attentions.⁸

A multifunctional fluorescent chemosensor by modulating the selectivity to various metal ions with the change of the tested media appears to be particularly attractive because of its convenience in real application. Sensitive probes have been designed to selectively differentiate Pb²⁺ and Hg²⁺, Au³⁺ and Hg²⁺, Zn²⁺ and Cu²⁺, and Cd²⁺, Hg²⁺ and Pb²⁺ via simple changes of different testing media.^{9–12} These studies demonstrated that solvent effect has a significant influence on detecting selectivity of chemosensors.

Recently, Duan reported a sugar–rhodamine fluorescence probe **1** that could selectively detect Hg²⁺ in natural water and living cells.¹³ The introduction of a sugar residue in probe **1** can improve both water solubility and bio-compatibility, thus increasing colorimetric and fluorescent “turn-on” selectivity, and sensitivity toward Hg²⁺ in 100% water and in cellular systems as well. Carbohydrate moieties, containing multi-OH groups, are believed to be good receptors for metal ions, and thus it is reasonable to expect that the probe **1** provides different selectivity through changing the solvent. Herein, we disclose that the sugar–rhodamine fluorescence probe **1** (Scheme 1) would exhibit a highly selective and sensitive fluorescence enhancement responding to Cu²⁺ ion.

2. Results and discussion

As mentioned in a previous report the synthesized probe **1** has high aqueous solubility and can completely dissolve in 100% pure water without adding any toxic organic co-solvent, which was used to detect Hg²⁺ ion in pure water with the fluorescent method.¹³ However, a very interesting phenomenon was found in our experiment, that is, the fluorescence signal of probe **1** to Hg²⁺ was significantly quenched by adding acetonitrile (CH₃CN) into aqueous media (Fig. 1a). Instead, the probe **1** exhibited a strong fluorescent signal to Cu²⁺, and the fluorescence intensity reached maximum when the ratio of acetonitrile to water was 20/80 (v/v) (Fig. 1b). As shown in three-dimensional excitation emission matrix fluorescence spectroscopy (3D-EEM) (Fig. S1†), the excitation and emission wavelengths of probe **1**-Cu²⁺ were at 520 nm/560 nm. A variation of pH from 5.8 to 9.8 does not cause significant changes in fluorescence intensity (Fig. 2), which

Scheme 1 Possible reaction mechanism of probe **1** with Cu²⁺.

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Fig. 1 The variation of fluorescence intensity ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 520/560$ nm) of probe **1** (10 μM) in the presence of (a) 10 μM Hg²⁺ and (b) 10 μM Cu²⁺ with different contents of co-solvent CH₃CN in aqueous media.

Fig. 2 The variation of fluorescence intensity ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 520/560$ nm) of probe **1** (10 μM) in water containing 20% of CH₃CN over a pH range of 4 to 10 with and without addition of 10 μM Cu²⁺.

implies that the probe **1** itself is relatively stable and of potential usage in aqueous and biological systems. The solution of probe **1** (10 μM) in acetonitrile/water (20/80, v/v) itself is colorless and non-fluorescent at a wavelength of 400 nm to 700 nm (Fig. 3a). However, a strong fluorescence signal (*ca.* 120 times to that of probe **1** alone) could be detected at $\lambda_{\text{ex}}/\lambda_{\text{em}} = 520/560$ nm when Cu²⁺ (20 μM in water containing 20% of acetonitrile) was added, ascribed to the generation of compound **3**. Moreover, a pink color could be observed by “naked-eye” simultaneously (Fig. 3b). The fluorescent signal reached maximum after a 30 min reaction period at room temperature (Fig. S2†). The changes of fluorescence intensity under various pH values were investigated (Fig. 2), and the suitable pH value is determined from 7.0 to 8.3.

Addition of Cu²⁺ chelating agents, such as EDTA (*ca.* 20 μM), into the detecting system, did not decrease the intensity of the fluorescence signals and visible color of probe **1**-Cu²⁺. These results suggest that the response of probe **1** to Cu²⁺ is an irreversible cation-catalyzed reaction (Scheme 1). Importantly, the

Fig. 3 (a) Fluorescence spectra ($\lambda_{\text{ex}} = 560$ nm) and (b) naked-eye color changes of probe **1** (10 μM) containing 20% (v/v) of CH₃CN before and after addition of Cu²⁺ (10 μM) at room temperature.

Fig. 4 Fluorescence intensity change ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 520/560$ nm, slit widths 1.0 nm) of probe **1** (10 μM in water containing 20% of acetonitrile) as a function of equivalent of Cu²⁺ after 30 min for each addition at room temperature.

titration of probe **1** with Cu²⁺ (from 1 to 7 equiv.) showed saturation behavior at 2 equiv. of Cu²⁺ (Fig. 4). In order to explore the reaction mechanism of the present system, detailed MALDI-TOF mass spectral analyses were conducted (Fig. S3 and S4†). In a short 5 min reaction time, three major peaks at *m/z* 591.3, 429.2 and 415.2 were observed, which were identical to the remaining probe **1** (calcd *m/z* 591.3 [M + H]⁺), compound **2** (calcd *m/z* 429.2 [M + H]⁺) and compound **3** (calcd *m/z* 415.2 [M + H]⁺). By elongating the reaction time to 30 min only the peak at *m/z* 415.2 corresponding to compound **3** was observed.

To evaluate the selectivity of probe **1**, eleven of various cations (including heavy metal ions that not only coexist but also often have a similar reactivity) were examined in parallel under the same working conditions. As shown in Fig. 5, the reaction of probe **1** (10 μM) with Cu²⁺ (10 μM) produced strong fluorescence response at $\lambda_{\text{em}} = 560$ nm, while the other cations did not exhibit the similar behavior even when their concentrations were set up at hundred times of Cu²⁺ (1.0 mM). This result indicates that the probe **1** has high specificity toward Cu²⁺ under the working conditions.

Fig. 6 shows the emission fluorescence spectra of probe **1** *vs.* Cu²⁺ contents. When the Cu²⁺ concentration increased from 0 to 10 μM while exciting at 520 nm, the emission fluorescence intensity of probe **1** at 560 nm increased more than 120 fold. A satisfactory linear relationship between fluorescence intensity and Cu²⁺ concentration was observed with the correlation coefficient as high as 0.9974, the linear relationship was still 0.9965 even when the Cu²⁺ concentration ranged from 0 to 1.0 μM .

Fig. 5 Fluorescence spectra ($\lambda_{\text{ex}} = 560$ nm, with slit widths 1.0 nm) of probe **1** (10 μM in water containing 20% of CH₃CN) upon addition of various metal ions. Solid line: probe **1** + Cu²⁺ (10 μM in water containing 20% of CH₃CN), dotted line: probe **1** + metal cation (1.0 mM in water containing 20% of CH₃CN).

Fig. 6 (a) Fluorescence titration spectra ($\lambda_{\text{ex}} = 520$ nm, with slit widths 1.0 nm) of probe **1** (10 μM in water containing 20% of CH_3CN) with Cu^{2+} from 0 to 10 μM at room temperature. (b) The plot of fluorescence intensity change ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 520/560$ nm, slit widths 1.0 nm) of probe **1** (10 μM in water containing 20% of CH_3CN) against varied concentrations of Cu^{2+} from 0 to 10 μM at room temperature. (Inset) Dependence of fluorescence intensity at 560 nm with respect to concentrations of Cu^{2+} ions ranging from 0 to 1.0 μM .

Similarly, the UV absorbance at 520 nm enhanced from 0.001 to 0.17 when increasing the concentration of Cu^{2+} from 0 to 10 μM (Fig. S5†). A satisfactory linear relationship between UV absorbance and Cu^{2+} concentration was observed with the correlation coefficient as high as 0.9940.

Furthermore, the detection limit of Cu^{2+} by the fluorometric method with the probe **1** was determined using both the S/N ratio test method and serial dilution method. It was found that the detection limit was as low as 0.20 μM (12 $\mu\text{g L}^{-1}$), the relative standard deviation of eleven runs was 5.1%. The detection limit (12 $\mu\text{g L}^{-1}$) of probe **1** to Cu^{2+} is *ca.* 160 times lower than the recommended water quality standard of Cu^{2+} (2.0 mg L^{-1}) for drinking water by WHO,⁶ EU,¹⁴ and Australia.¹⁵ The present result implies that the 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+} . Fig. 7 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 the naked-eye when the Cu^{2+}

Fig. 7 “Naked-eye” color changes of probe **1** (0.5 equiv. to Cu^{2+}) in water containing 20% of CH_3CN under natural light upon addition of different concentrations of Cu^{2+} (from 0 to 50 μM) at room temperature.

Fig. 8 The prepared color chart according to the solution color with different concentrations of Cu^{2+} ions.

concentration increased to 3 μM (*ca.* 0.20 mg L^{-1}), 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 used conveniently to screen the Cu^{2+} concentration in the sample with the 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 Fig. 8, the Cu^{2+} concentration in water sample could be identified through comparing the color in the test vial and chart with the naked eye.

3. Conclusions

We have investigated the properties of the fluorescent probe **1** for Cu^{2+} detection based on a Cu^{2+} triggered ring-opening reaction of spirolactam in aqueous medium. The probe **1** is a dual-responsive fluorescent and colorimetric Cu^{2+} -specific sensor in aqueous media. Fluorescent probe **1** exhibited an excellent Cu^{2+} ions selectivity over other cations, as well as a satisfactory detection limit at 12 $\mu\text{g L}^{-1}$. More importantly, the probe displays significant naked-eye color changes as the Cu^{2+} concentration increases, the formed pink color can be obviously recognized by naked eye even at 3 μM (*ca.* 0.20 mg L^{-1}) of Cu^{2+} , which is about ten times lower than the WHO recommended value for Cu^{2+} (2.0 mg L^{-1}) in drinking water. A color chart was prepared according to the correlation of the color appeared and the concentration of Cu^{2+} , providing 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. The most interesting is that the same chemosensor can be successfully applied to detect Hg^{2+} and Cu^{2+} by controlling test media. The theme of the molecular design presented here may help the development of more efficient rhodamine-based chemosensor platforms.

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