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A novel fluorescein derivative as a colorimetric chemosensor for detecting copper(II) ion

Tianrong Li, Zhengyin Yang*, Yong Li, Zengchen Liu, Gaofei Qi, Baodui Wang

College of Chemistry and Chemical Engineering and State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, PR China

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ABSTRACT

A novel fluorescein derivative, synthesized by the reaction of fluorescein hydrazide and 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone, was evaluated as a chemoselective metal ion sensor. Addition of Cu^{2+} to an aqueous solution of the fluorescein derivative resulted in a rapid color change from colorless to deep yellow together with a distinctive change in UV–vis absorption spectrum. However, other common alkali-, alkaline earth-, transition- and rare earth metal ions induced no or minimal spectral changes. The stoichiometry of the reaction and association constant of the fluorescein derivative with Cu^{2+} are described. Experimental results indicate that the fluorescein derivative could provide a rapid, selective and sensitive response to Cu^{2+}, and could be used as a potential Cu^{2+} colorimetric chemosensor in aqueous solution.

1. Introduction

Metal ion sensors are employed in applications ranging from clinical toxicology, environmental bioinorganic chemistry, bioremediation, and waste management [1–3] and much attention has focussed to the development of sensing devices for copper(II). As the third most abundant divalent metal ion in the human body, after Fe^{2+} and Zn^{2+}, Cu^{2+} plays a pivotal role in a variety of fundamental physiological processes in organisms ranging from bacteria to mammals [4,5]. Copper compounds are also employed for plant diseases treatment, water treatment and as preservatives for wood and leather. Nonetheless, while a low-level background intake of copper is indispensable, high doses of copper can be harmful and even toxic to biological systems [4,6].

Many sensing methods for detecting Cu^{2+} have been described, such as colorimetric and fluorescent chemosensors, and electrochemical methods [7–9]. Colorimetric sensors are promising due to the simplicity of the assay. Furthermore, colorimetric assays have a significantly lower capital cost than closely related methods, such as fluorescent sensors, for which both spectrophotometric equipment and a UV light source are required [10–16].

The fluorescein and rhodamine family of dyes, with spirolactam structure (closed form) are non-fluorescent. As shown in Fig. 1, ring-opening of the spirolactam gives the open form and results in a strong fluorescence and obvious color change [17–20]. Due to large visible-range extinction coefficients and high fluorescence quantum yields for fluorescein and rhodamine, these compounds are excellent antenna chromophores [21–26].

In recent years, several rhodamine-based chemosensors and chemodosimeters for metal ions, such as Cu^{2+} [27–30], Hg^{2+} [20,31–34], Fe^{3+} [35], and Pb^{2+} [19] have been studied. The cation-sensing mechanism of these probes is based on the change in structure between spirocyclic and opencyclic forms. However, fluorescein-based probes have received comparatively little attention [36,37]. We synthesized a fluorescein-based colorimetric chemosensor, 1-phenyl-3-methyl-5-hydroxypyrazole-4-benzoyl (fluorescein)hydrazone (**1**), for rapid, selective and sensitive response to Cu^{2+} in aqueous media (Fig. 2). Solutions of **1** are colorless, but upon addition of micromolar Cu^{2+} a deep yellow color is obtained. Addition of other common alkali-, alkaline earth-, transition- and rare earth metal ions result in no or minimal spectral change. Compound **1** is a naked-eye chemosensor for

* Corresponding author. Tel.: +86 931 891 3515; fax: +86 931 891 2582.
E-mail address: yangzy@lzu.edu.cn (Z. Yang).

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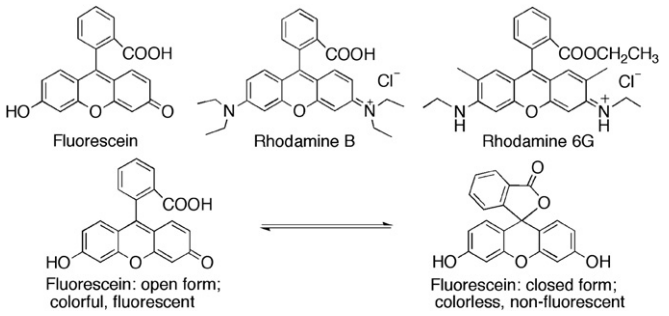

Fig. 1. Structure of fluorescein, rhodamine B and rhodamine 6G.

detection of Cu^{2+} that upon chelation of Cu^{2+} **1** will change to a strongly colored ring-opened form.

2. Experimental

2.1. Materials and instruments

All the materials for synthesis were purchased from commercial suppliers and used without further purification. With the exception of $Al(NO_3)_3$ and $MgSO_4$, aqueous solutions of metal ions were prepared from their chloride salts.

NMR spectra were taken on a Bruker Avance DRX-200 or Varian Mercury Plus-300 BB spectrometer with TMS (tetramethylsilane) as internal standard and DMSO-d_6 as solvent. Mass spectra were obtained on a Bruker Esquire 6000 spectrometer. UV–vis absorption spectra were obtained with a Perkin Elmer Lambda 35 UV–vis spectrophotometer and recorded in quartz cells with 1 cm optical path length. Fluorescence spectra were recorded on a Hitachi RF-4500 fluorescence spectrophotometer.

2.2. General procedures of metal ion sensing

2.2.1. Job's plot analyses

For Job's plot analyses (in Section 3.3), a 1.0×10^{-3} M stock solution of Cu^{2+} in H_2O, and 1.0×10^{-3} M stock solution of **1** in DMSO (dimethylsulfoxide) were prepared. The Cu^{2+} stock solution and the stock solution of **1** were placed in a test tube to obtain a total volume of 20 μL, then diluted to 2 mL with DMSO/H_2O (4:6, v/v) to get the test solution (nine test solutions were got; for the first test solution, 2 μL stock solution of Cu^{2+} and 18 μL stock solution of **1** were used, then diluted to 2 mL with DMSO/H_2O (4:6, v/v) to get the first test solution; for the second test solution, 4 μL stock solution of Cu^{2+} and 16 μL stock solution of **1** were used; for the third test solution, 6 μL stock solution of Cu^{2+} and 14 μL stock solution of **1** were used; until 18 μL stock solution of Cu^{2+} and 2 μL stock solution of **1** were used to get the ninth test solution). All the analyses were performed with $[\mathbf{1}] + [Cu^{2+}] = 10.0$ μM. The absorbance at 495 nm of these test solutions was recorded.

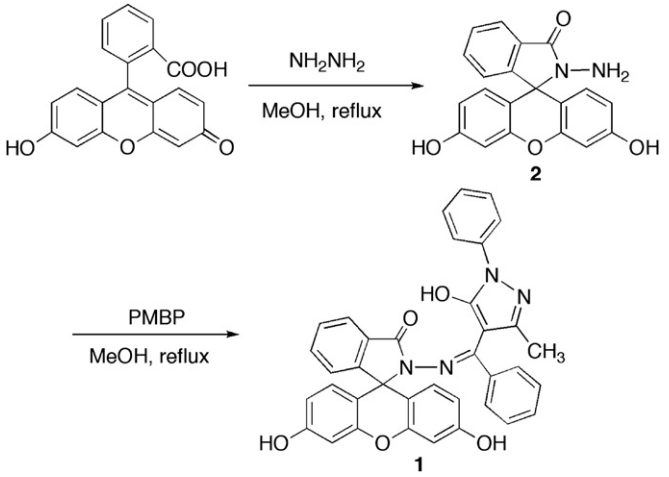

Fig. 2. Synthetic route of chemosensor **1**.

2.2.2. Spectral analyses

In other spectral analyses, a 1.0×10^{-3} M solution of **1** was prepared in DMSO, and then diluted by DMSO/H_2O (4:6, v/v) to give a 1.0×10^{-5} M stock solution of **1** for spectral analyses. Stock solutions of metal ions were prepared in H_2O (2.0×10^{-3} M of Cu^{2+} was used in Section 3.2; 2.0×10^{-2} M of metal ions were used in other sections, except Sections 3.2 and 3.3). Each time, a 2 ml stock solution of **1** (1.0×10^{-5} M) was added to the quartz cell, and the required quantity of stock solution of metal ions was added with a microsyringe.

All the measurements were taken at room temperature about 298 K. After 2 min of the mixing of metal ions with **1**, UV–vis absorption spectra or fluorescence emission spectra were measured, unless otherwise indicated. For all fluorescent tests, excitation wavelength was 287 nm, with excitation and emission slit widths both 5 nm. Addition of metal ions increased the volume by no more than 0.04 mL, so that dilution was insignificant.

2.3. Synthesis

2.3.1. Synthesis of fluorescein hydrazide (*2*)

In a 100 mL flask containing a suspension of fluorescein (6 g, 18.1 mmol) in 50 mL methanol, excess hydrazine hydrate (24 mL; hydrazine content >80 mass%) was added. The reaction mixture was heated to reflux for 7 h with stirring, during which time the suspended particles were consumed and a clear solution was obtained. The ensuing solution was allowed to cool and poured into 400 mL H_2O at which time, a yellow precipitated formed immediately, which was allowed to settle for 2 h. The aqueous suspension was filtered, washed with water until the filtrate was colorless, and washed 3 × 10 mL with cold absolute ethanol. The crude product was purified by recrystallization from ethanol to give 3.59 g of **2** as an off-white solid (57%). Melting point: 262–264 °C. ^{1}H NMR (200 MHz, DMSO-d_6), δ (ppm): 4.38 (s, 2H), 6.42 (m, 4H), 6.59 (s, 2H), 6.97 (m, 1H), 7.48 (m, 2H), 7.76 (m, 1H), 9.81 (s, 2H). ^{13}C NMR (50 MHz, DMSO-d_6), δ (ppm): 64.6, 102.3, 109.9, 111.9, 122.3, 127.9, 128.4, 129.3, 132.6, 151.5, 152.4, 158.1, 165.4.

2.3.2. Synthesis of 1-phenyl-3-methyl-5-hydroxypyrazole-4-benzoyl(fluorescein)hydrazone (*1*)

In a 25 mL flask, **2** (0.346 g, 1 mmol) and 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (PMBP; 0.278 g, 1 mmol) were suspended in 10 mL methanol. The mixture was refluxed for 8 h with stirring, during which time a clear solution formed. Following reaction, the mixture was allowed to cool to room temperature and a yellow precipitate formed. The precipitated was separated by filtration and washed with 3 × 10 mL methanol. After drying, 0.25 g of **1** (bright yellow solid) was prepared in 41% yield. Melting point >320 °C. ^{1}H NMR (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 3H), 5.96 (s, 2H), 6.35 (s, 2H), 6.61 (d, J = 2.1 Hz, 3H), 7.12 (m, 3H), 7.21 (s, 2H), 7.36 (m, 3H), 7.63 (m, 2H), 7.77 (d, J = 8.1 Hz, 2H), 7.89 (d, J = 6.9 Hz, 1H), 10.04 (s, 2H), 11.51 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6), δ (ppm): 15.1, 66.4, 100.7, 102.4, 107.5, 113.2, 118.2, 123.3, 124.4, 127.1, 127.7, 128.7, 129.5, 130.2, 134.7, 138.3, 147.4, 149.6, 152.8, 159.0, 164.7,