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A new rhodamine derivative-based chemosensor for highly selective and sensitive determination of Cu^{2+}

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ABSTRACT

A new rhodamine derivative (R1) has been synthesized by a hydrazone formation of rhodamine B hydrazide with pyrrole-2-carboxaldehyde and its binding affinity to metal ions were examined. R1 shows highly binding selectivity to Cu^{2+} over commonly coexistent metal ions in neutral aqueous-organic media including alkali, alkaline-earth and transition metals. The linear response to Cu^{2+} was obtained across the concentration range of 0.4–10 μM with the detection limit of 280 nM. Using newly synthesized probe R1, determination of Cu^{2+} concentration in drinking water and serums, and living cell imaging of Cu^{2+} were carried out. Also, by incorporating the R1 with filter paper, the sensor was performed in Cu^{2+} spiked samples.

1. Introduction

Copper is an essential trace element in biological systems of plants, living cells, and humans [1–3]. However, at high concentrations, it causes oxidative stress and disorders associated with neurodegenerative diseases for humans, such as Alzheimer's disease, Wilson's disease, and Menke's disease [4]. US-EPA advises the maximum contaminant level goal (MCLG) for copper is 1.3 mg L^{-1} in drinking water, exceeding the value may cause potential health problem [5]. Several methods have been used to detect metal ions such as atomic absorption spectrometry (AAS) [6,7], inductive coupled mass atomic emission spectrometry (ICP-AES) [8], inductive coupled plasma mass spectroscopy (ICP-MS) [9], plasmon resonance Rayleigh scattering (PRRS) spectroscopy [10], and electrochemical methods [11,12]. However, they are rather complicated by pretreatment procedures, time-consuming analysis, costly instruments, and the fact that they are unable to be used in the field. Fluorescent chemosensors for transition-metal ions have attracted much attention due to their implicitness, high selectivity and sensitivity, low cost, and real-time monitoring [13]. For the determination of Cu^{2+}, almost previous works have reported the "on-off" fluorescence probes [14–17]. This was due to its paramagneticity of the Cu^{2+} ion ($3d^9$), leading to the fluorescence quenching of the Cu^{2+}-probe complex. The quenching behavior of the probe, however, is not as sensitive as a fluorescence enhancement response in terms of signal detection. Therefore, the development of a turn-on fluorescence probe is of interest.

Rhodamine fluorophore and its derivatives have been extensively studied as turn-on type chemosensors due to their excellent photophysical properties such as the long absorption and emission wavelength, high fluorescence quantum yield, large extinction coefficient, and high photostability [18]. The rhodamine derivatives with an opened spirolactum ring (effecting from metal ion chelation) give off a strong fluorescent emission and pink color [19]. Based on this mechanism, fluorescent enhancement of rhodamine probes have been reported for Cu^{2+} detection [20–24]. However, these chemosensors are disadvantageous from other metal ions interferences, long response times, and complicated procedures for synthesis [25–30]. Recently, the furan-bound rhodamine derivative was reported as Cu^{2+} selective chemosensor with high selectivity of Cu^{2+} in living cells [31]. However, this furan derivative showed the low binding affinity with Cu^{2+} and the interference effect of Cu^{2+} with Hg^{2+}. Instead of furan, we are interested to use pyrrole as a linker because N atom of pyrrole makes high electrostatic contributions to the binding of transition metal ions better than O atom of furan [32]. Also, Cu^{2+} is able to chelate to form a bidentate

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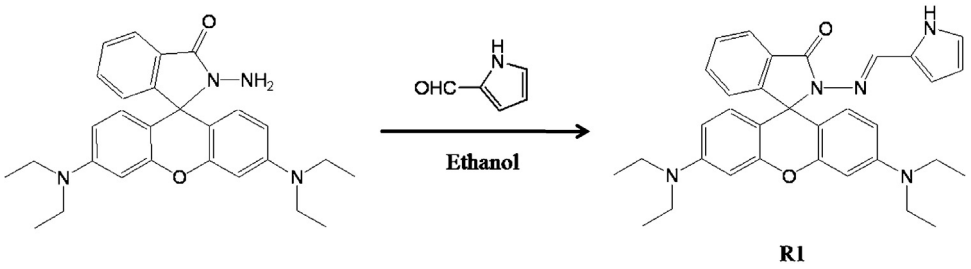

Scheme 1. Synthesis of **R1**.

molecular structure via carbonyl O atom of the xanthenes group and N atom of pyrrole; expectedly a better selectivity toward Cu^{2+}.

Herein, we designed and synthesized a new rhodamine derivative bearing a pyrrole unit as a fluorogenic and chromogenic sensor for Cu^{2+}. Among the various metal ions, this probe exhibits remarkably enhanced absorbance intensity and shows significant turn-on fluorescence intensity for Cu^{2+} in HEPES/acetonitrile buffer at physiological condition. In addition, the probe has been successfully applied for rapid and sensitive measuring for Cu^{2+} in drinking water, human serum samples and in vivo imaging of HeLa cells. In addition, the probe was demonstrated in a form of paper-based sensor for applying in real samples.

2. Experimental

2.1. Reagents and instruments

Rhodamine B, hydrazide hydrate, pyrrole-2-carboxaldehyde, and 2-[4-(2-hydroxyehtyl) piperazin-1-yl] ethanesulfonic acid (HEPES) were purchased from Sigma–Aldrich. Acetonitrile (Carlo Erba) and double-distilled water were used throughout the experiment. Solutions of Ba^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+} and Ni^{2+} were prepared from their chloride salts and solutions of Ag^{+}, Cd^{2+}, Cr^{6+}, Fe^{2+}, $Pb^{2+}$and Zn^{2+} were prepared from their nitrate salts. Thin layer chromatography (TLC) was carried out using silica gel 60 F254 (Merck) and column chromatography was conducted over silica gel 60.

NMR spectra were recorded on a Bruker 400 MHz spectrophotometer. Mass spectra were obtained on a Bruker microTOF LC-ESI spectrometer. Absorption spectra were measured on a GCB Cintra 404 UV-Vis spectrometer. Fluorescence spectra measurements were recorded on a JASCO FP-6500 spectrofluorometer. All measurements were operated at a room temperature of ~298 K.

2.2. Synthesis of *R1*

Rhodamine B hydrazide was prepared according to the literature [33]. Rhodamine B hydrazide (1.0 mmol, 0.4560 g) and pyrrole-2-carboxaldehyde (1.2 mmol, 0.1141 g) were mixed in boiling ethanol with the addition of 3 drops of acetic acid (Scheme 1). After 24 h of stirring, the reaction progress was monitored by TLC. After the reaction was completed, the reaction solution was cooled down to room temperature and poured into a brine solution before extracting with dichloromethane. The combined organic extracts were dried over anhydrous sodium sulfate before being filtered and the solvent was completely evaporated. The crude solid product was further purified by silica gel column chromatography [hexane/ethyl acetate (3/2, v/v)] before the desired **R1** was obtained as a colorless solid in 45% yield. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.40 (s, 1H), 7.92–7.99 (d, J = 7.1 Hz, 1H), 7.40–7.48 (m, 2H), 7.08–7.12 (d, J = 7.0 Hz, 1H), 6.70 (s, 1H), 6.49–6.53 (d, J = 9.0 Hz, 2H), 6.39–6.42 (d, J = 2.0 Hz, 2H), 6.22–6.28 (m, 3H), 6.01–6.03 (m, 1H), 3.37–3.48 (q, J = 7.0 Hz, 8H), 1.15–2.01 (t, J = 7.0 Hz, 12H). ^{13}C NMR (400 MHz, $CDCl_3$), δ (ppm): 164.52, 152.49, 151.63, 148.48, 138.49, 132.69, 128.55, 128.31, 127.75, 127.51, 123.16, 122.70, 120.71, 113.32, 109.02, 107.62, 105.29, 97.40, 65.37, 43.88, 12.19. HRMS m/z calcd. for $C_{33}H_{35}N_5O_2$: 533.2791 found: 534.2858 [**R1**+H]$^{+}$.

2.3. Measurement procedures

2.3.1. General procedure for Cu^{2+} determination

A 1 mM stock solution of **R1** was prepared by dissolving **R1** in absolute acetonitrile. A standard stock solution of Cu^{2+} ion (10 mM) was prepared by dissolving an appropriate amount of copper chloride in Milli-Q water. The complex solution of Cu^{2+}/**R1** was prepared by adding 100 μL of stock solution of **R1** and 100 μL of the stock solution of Cu^{2+} in a volumetric flask (10 mL). After adjusting the final volume with acetonitrile/HEPES buffer (10 mM, pH 7.0, 1:1 of v/v), the solution was placed at room temperature for 30 min. After that, the solution was measured with fluorescence spectroscopy. The excitation wavelength was performed at 510 nm. Both excitation and emission slit widths were 3 nm. All solutions were protected from light and kept at 4 °C for further use. A blank solution of **R1** was prepared under the same conditions without Cu^{2+}. Stock solutions of other metal ions were prepared in water with a similar procedure.

2.3.2. Detection of Cu^{2+} in human serums

The human serum samples were treated with 0.05% HNO_3. After that, the serum samples were centrifuged and the protein components were removed. The supernatant solution was collected and kept at 4 °C. Next, the supernatant serum was transferred and mixed with **R1** solution in a 10 mL volumetric flask. Acetonitrile/HEPES buffer (10 mM, pH 7.0, 1:1 of v/v) was added to adjust to the final volume of 10 mL. The solution was left at room temperature for 30 min before measuring with a fluorescence spectrophotometer with 510 nm of excitation wavelength.

2.3.3. Cell culturing and imaging

Living HeLa cells (cervical cancer cells) were provided by the National Center for Genetic Engineering and Biotechnology (Thailand). Cells were grown in EMEM supplemented with 10% FBS (fetal bovine serum), 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM essential amino acids and 1.5 g/L sodium bicarbonate in an atmosphere of 5% CO_2 and 95% air at 37 °C. Cells were seeded on a 96-well plate at 5×10^5 cells per well and allowed to adhere for 24 h. Immediately before the experiments, the cells were washed with phosphate-buffered saline (PBS) three times and then incubated with 20 μM of **R1** (in the culture medium) for 20 min at 37 °C. The **R1** probe was taken up into cells by endocytosis mechanism [34,35]. After washing with PBS (three times) to remove the remaining **R1**, the treated cells were incubated with 50 μM $CuCl_2$ (in the culture medium) for 20 min at 37 °C. The remaining copper ions were removed by washing with PBS three times before adding 200 μL of PBS into the well. Fluorescent imaging was performed with an Olympus IX71 inverted fluorescence microscope to record