Tetrahedron Letters 56 (2015) 4293–4298

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A phenothiazine–rhodamine ratiometric fluorescent probe for Hg^{2+} based on FRET and ICT

Wenqi Zhao, Xingliang Liu, Haitang Lv, Hua Fu, Yan Yang, Ziping Huang *, Aixia Han *

Chemical Engineering College, Qinghai University, Xining 810016, China

ARTICLE INFO

Article history:
Received 13 March 2015
Revised 9 May 2015
Accepted 12 May 2015
Available online 27 May 2015

Keywords:
Ratiometric sensing
FRET
ICT
Hg^{2+}
Fluorescence probe

ABSTRACT

Rhodamine B and phenothiazine building blocks have been used to synthesize a novel ratiometric fluorescent probe **1** for Hg^{2+}. Based on fluorescence resonance energy transfer (FRET) and internal charge transfer (ICT) mechanisms, a low detection limit for Hg^{2+} of 1.8 ppb has been achieved. The intensity ratio (F580 nm/F520 nm) of fluorescent probe **1** increased on going from 0.1 to 1.0 equiv of Hg^{2+}. In the FRET process, energy is transferred from the phenothiazine moiety to the rhodamine B moiety. Probe **1** shows a selective response to Hg^{2+} by color and fluorescence changes.

Introduction

Fluorescent probes for detecting heavy metal ions in environmental and biological systems have received considerable attention.[1,2] Mercury, one of the major and most dangerous contaminant metal ions, can exert long-term contaminative effects and cause serious health problems due to its ability to pass through biological membranes.[3] Hence, it is highly desirable to design, synthesize, and apply fluorescent sensors with high sensitivity and selectivity for Hg^{2+}.

Some fluorescent probes have been synthesized and used in biosensing, bioimaging, and environmental detection.[4] However, most of these probes have limitations in that they measure only decreases or increases in fluorescence intensity. The location, variations in concentration, and environment of the probe may change the emission collection efficiency and excitation intensity, which may further influence the accuracy and reliability of measurements.[5,6] In order to overcome these influences, ratiometric fluorescent probes have been developed.[7] Ratiometric fluorescent probes measure changes in the ratio of emission intensities at two different wavelengths. In this context, two major mechanisms have been developed for sensing applications. Of these, fluorescence resonance energy transfer (FRET) is more advantageous than internal charge transfer (ICT)[8] and has become one of the most widely used sensing mechanisms, most notably in the rhodamine-based FRET system.[9–14]

Rhodamine dyes have excellent photophysical properties, such as high fluorescence quantum yields, and large absorption coefficients.[15] The rhodamine fluorophore generally acts as the energy acceptor in FRET systems. As a result, red shifts of their absorption and emission spectra to a longer wavelength (ca. 580 nm) are observed. As donor components, *N*-dansyl thiophene,[16] naphthalimine,[17] BODIPY,[18] and coumarin[19] are widely used because there are strong overlaps between their emission spectra and the absorption spectrum of rhodamine. Some FRET-based ratiometric fluorescent probes specifically for Hg^{2+} have been developed,[10,12,20–25] most of which are based on Hg^{2+}-promoted spiro-opening of rhodamine. On-site and remote detections and even 'naked-eye' detections have been achieved.[26–28] In addition, the linear fluorescence responses of these probes have been further exploited for the quantification of Hg^{2+}. The detection limits were located within the range of ppb to tens of ppb.[29–31] However, the majority of published ratiometric Hg^{2+} probes have been irreversible and have lacked selectivity due to interference from other metal ions, particularly Cu^{2+}, Ag^{+}, and Pb^{2+},[25,32–36] which induce similar ring-opening reactions as Hg^{2+}.

Inspired by the above achievements, we have designed a novel ratiometric fluorescent probe **1** consisting of a phenothiazine donor and a rhodamine acceptor, for the sensitive and selective detection of Hg^{2+}.

In the absence of Hg^{2+} ions, the fluorescent probe **1** is in a 'FRET-OFF' state. On excitation with light of wavelength 415 nm, an

* Corresponding authors. Tel./fax: +86 971 531 0427.
E-mail addresses: hanax@qhu.edu.cn (Z. Huang), hanaixia@tsinghua.org.cn (A. Han).

http://dx.doi.org/10.1016/j.tetlet.2015.05.045
0040-4039/

emission from the donor appears at 550 nm. In the presence of Hg^{2+}, the FRET process occurs and a new emission of rhodamine B develops at 580 nm, leading to a significant color change from light-yellow to pink. In this way, the fluorescent probe **1** can be used for the ratiometric detection of Hg^{2+} ions with a low detection limit of 1.8 ppb.

Materials and methods

Materials

All chemicals and solvents were of analytical grade and were used without further purification. DMF was distilled from P_2O_5 prior to use. The silica gel used was 300–400 mesh. Compounds **2**,[37] **3**,[38] and **6**[39] were synthesized according to literature procedures. ^{1}H NMR spectra were recorded on a Mercury Plus instrument at 400 MHz using $CDCl_3$ as the solvent in all cases. Mass spectra were recorded on an Agilent Technologies 6224 spectrometer. UV/vis spectra were acquired on a Shimadzu UV-2550 spectrophotometer. Fluorescence measurements were performed on a Cary Eclipse fluorescence spectrophotometer.

Synthetic routes

Synthesis of 4

Compound **3** (6.3 g, 17.8 mmol) was added to NaH (2.8 g, 116.7 mmol) in DMSO (70 mL), and the mixture was stirred at 0 °C for 0.5 h. 3-Phenyl-2-azirine (2.1 g, 17.8 mmol) was then added and the resulting mixture was stirred for 4 h at room temperature. The solution was then poured into distilled water and extracted with dichloromethane. The residue obtained after work-up was purified by column chromatography eluting with dichloromethane. The crude product was recrystallized from dichloromethane and petroleum ether, yielding compound **4** as a yellow powder (2.8 g, yield: 35%). ^{1}H NMR ($CDCl_3$, δ, ppm): 8.34 (s, 1H), 7.57 (d, J = 7.2 Hz, 2H), 7.37 (t, J = 7.8 Hz, 3H), 7.23–7.09 (m, 5H), 6.96–6.84 (m, 3H), 6.73 (d, J = 1.6 Hz, 1H), 3.87–3.83 (t, J = 6.6 Hz, 2H), 1.86–1.79 (m, 2H), 1.48–1.28 (m, 10H), 0.91–0.87 (m, 3H) (Fig. S1, Supplementary material). TOFMS: $[M+H]^+$: 452.2268; found: 453.2355 (Fig. S2, Supplementary material).

Synthesis of 5

$POCl_3$ (0.46 mL, 5.0 mmol) was added dropwise to dry DMF (0.38 mL, 5.0 mmol) at 0 °C and the mixture was stirred for 0.5 h. A solution of compound **4** (2.0 g, 4.4 mmol) in 1,2-dichloroethane (80 mL) was added dropwise to the mixture, keeping the temperature at 0 °C. The resulting mixture was then stirred for 3 h at room temperature. The solution was poured into distilled water, the pH was adjusted to 10 with 2 M NaOH, and the mixture was extracted with dichloromethane. The residue obtained after work-up was purified by column chromatography eluting with dichloromethane to afford a yellow powder (1.3 g, yield: 58%). ^{1}H NMR ($CDCl_3$, δ, ppm): 10.01 (s, 1H), 9.63 (d, J = 5.6 Hz, 1H), 7.57–7.40 (m, 7H), 7.20–7.13 (m, 2H), 6.97–6.88 (m, 3H), 6.64 (d, J = 2.8 Hz, 1H), 3.87 (t, J = 6.6 Hz, 2H), 1.86–1.79 (m, 2H), 1.74–1.27 (m, 10H), 0.91–0.87 (m, 3H) (Fig. S3, Supplementary material). TOFMS: $[M+H]^+$: 480.2235; found: 481.2305 (Fig. S4, Supplementary material).

Synthesis of 1

Rhodamine B hydrazine **6** (0.37 g, 0.8 mmol) and compound **5** (0.35 g, 0.7 mmol) were dissolved in ethanol (20 mL). Acetic acid (ca. 3–5 drops) was added and the resulting mixture was heated under reflux overnight. The resulting solution was cooled to room temperature and the precipitate that separated was collected by filtration under reduced pressure. The residue was thoroughly washed with methanol to isolate **1** in pure form (0.6 g, yield: 90%). ^{1}H NMR ($CDCl_3$, δ, ppm): 10.60 (s, 1H), 8.05 (s, 1H), 7.99 (d, J = 6.0 Hz, 1H), 7.47–7.36 (m, 6H), 7.15–7.08 (m, 5H), 6.93–6.85 (m, 3H), 6.58 (d, J = 8.8 Hz, 2H), 6.45 (s, 1H), 6.36 (s, 2H), 2.26 (d, J = 8.4 Hz, 2H), 3.85 (t, J = 6.8 Hz, 2H), 3.35–3.24 (m, 8H), 3.17 (s, 1H), 1.83 (t, J = 6.6 Hz, 2H), 1.45 (s, 2H), 1.29 (d, J = 10.0 Hz, 8H), 1.12 (t, J = 6.8 Hz, 12H), 0.88 (d, J = 6.8 Hz, 3H) (Fig. S5, Supplementary material). TOF-MS: $[M+H]^+$: 918.4655; found: 919.4723 (Fig. S6, Supplementary material).

Procedures for ion detection

Ultrapure water was used throughout the experiments. Solutions of Hg^{2+}, Zn^{2+}, Ba^{2+}, Pb^{2+}, Ca^{2+}, Cu^{2+}, Mg^{2+}, Cd^{2+}, and Co^{2+} were prepared from their nitrate salts. A stock solution (1 mM) of probe **1** was prepared in acetonitrile. This stock solution of **1** was diluted to the requisite concentrations (10 μM and 20 μM) with acetonitrile. The 1 M Hg^{2+} stock solution was diluted to 0.25 mM and 0.5 mM with ultrapure water for spectrometric titration studies. In the titration experiments, a 2.5 mL aliquot of probe **1** solution (10 μM or 20 μM) was placed in a quartz optical cuvette of pathlength 1 cm, and Hg^{2+} solution was added gradually by means of a micro-pipette. Spectral data were recorded instantly after the addition of Hg^{2+} solution under excitation at 415 nm. Slit widths were 5 nm/5 nm for probe **1** at 10 μM; 2.5 nm/5 nm for probe **1** at 20 μM.

Results and discussion

Response mechanism of 1 to Hg^{2+}

Probe **1** was synthesized through a five-step route (Schemes 1 and 2). In the absence of Hg^{2+}, the rhodamine B moiety adopts a closed, non-fluorescent spirolactam form, so FRET is suppressed. In this case, only the emission of the phenothiazine donor (at 550 nm) is observed upon excitation of the phenothiazine donor (at 415 nm). In the presence of Hg^{2+}, a ring-opening reaction of the rhodamine B moiety is induced.[17] This ring-opening reaction involves coordination of the Hg^{2+} by a carbonyl oxygen atom, the pyrrole nitrogen atom, and two imino nitrogen atoms. At the same time, energy is transferred from the phenothiazine moiety to the rhodamine B moiety, and the FRET effect is restored, giving rise to pink fluorescence (Fig. 1). Based on this FRET, excitation of the phenothiazine moiety (at 415 nm) results in a strong red emission of the rhodamine B moiety (at 580 nm). Besides, the phenothiazine moiety is a 'push-pull' electron system, in which the pyrrole group acts as a donor and the phenothiazine moiety as an acceptor. In the presence of Hg^{2+}, due to coordination between the pyrrole nitrogen atom and Hg^{2+}, the 'push' effect of this nitrogen is weakened, and this results in a blue shift in the emission of the phenothiazine moiety from 550 nm to 520 nm (Fig. 5).

FRET efficiency

The spectral overlap is the main factor that determines the efficiency of FRET. The phenothiazine fluorophore was chosen as the energy donor in our FRET system because its broad emission (400–650 nm) covers a good part of the absorption spectrum of rhodamine. This design provides favorable conditions for FRET (Fig. 2). FRET efficiency (E) can be obtained by measuring the fluorescence intensities of the donor in the presence or absence of the acceptor.[40] Thus, E can be calculated by Eq. 1:

$$E = 1 - I_D/I_{D0} \quad (1)$$

where I_D and I_{D0} are the fluorescence intensities with and without the acceptor.