A New bis(rhodamine)-Based Fluorescent Chemosensor for Fe$^{3+}$

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Received: 17 September 2011 / Accepted: 29 November 2011 / Published online: 7 December 2011
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Abstract A new bis(rhodamine)-based fluorescent probe 4 was synthesized, and it exhibited high selectivity for Fe$^{3+}$ over other commonly coexistent metal ions in both 50% ethanol and Tris–HCl buffer. Upon the addition of Fe$^{3+}$, the spirocyclic ring of 4 was opened and a significant enhancement of visible color and fluorescence in the range of 500–600 nm was observed.

Keywords Fluorescent probe · Rhodamine · Fe$^{3+}$

Introduction

During the recent two decades, the design and synthesis of compounds for sensing environmentally and biologically relevant important ionic species, particularly for heavy metal and transition metal cations, is currently of great interest [1–5]. Numerous excellent works focus on the selective and sensitive detection of transition metal ions; e.g., detection of Cu$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, and Hg$^{2+}$ have been reported [6–15]. The trivalent form of iron is an essential element in man. It provides the oxygen-carrying capacity of heme and acts as a cofactor in many enzymatic reactions involved in the mitochondrial respiratory chain [16–18]. However, the development of new fluorescent Fe$^{3+}$ indicators, especially those that exhibit selective Fe$^{3+}$-amplified emission [19–22], is still a challenge. Therefore, there is an urgent need to develop chemical sensors that are capable of detecting the presence of iron in environmental and biological samples at a physiological pH value. On the other hand, rhodamine derivatives are nonfluorescent and colorless, whereas ring-opening of the corresponding spirolactam gives rise to strong fluorescence emission and a pink color [23–28]. Recently, some bis(rhodamine)-based fluorescent probe were reported for the detection of Fe$^{3+}$ metal ions [29–31]. In this paper, we report three new rhodamine-based turn-on fluorescent sensors 2–4 (Scheme 1) for Fe$^{3+}$. Sensor 4 shows very high sensitivity and selectivity toward Fe$^{3+}$ over other metal ions.

Experimental Section

All the materials for synthesis were purchased from commercial suppliers and used without further purification. The solutions of metal ions were prepared from their nitrate salts, except for FeCl$_2$, CrCl$_3$ and MnCl$_2$. The use of Fe(NO$_3$)$_3$ and FeCl$_3$ yielded nearly the same results. Tris–HCl buffer solutions (pH=7.15) were prepared using 0.01 M Tris, 0.1 M KNO$_3$ and proper amount of HCl (about 0.01 M).
Rhodamine B derivative (1) was synthesized according to the literature [1–5]. A Hitachi F-4500 spectrofluorimeter was used for fluorescence measurements. The absorption spectra were recorded with a Techcomp UV-8500 spectrophotometer (Shanghai, China). NMR spectra were measured on a Bruker AMX-400 spectrometer at 400 MHz in CDCl₃. Elemental analyses were carried out with a Flash EA 1112 instrument. Mass spectra were acquired in positive ion mode using a Bruker ESQUIRE 3000 ion trap spectrometer equipped with a gas nebulizer probe, capable of analyzing ions up to m/z 6000.

**Synthesis of 2**

Benzoyl chloride (0.140 g, 1 mmol) was slowly added into the mixture of 1 (0.484 g, 1 mmol) and triethylamine (0.5 mL) in 20 mL CH₂Cl₂ at 0 °C. After the addition, the mixture was stirred at room temperature for 5 h. The solvent was removed and the residue was purified by silica gel column chromatography with EtOAc/PE (4/1, v/v) as eluent to afford 4, yield 98.5%; ¹H NMR (400 MHz, CDCl₃, δ ppm): 8.33 (s, 1H), 7.96 (m, 3H), 7.47 (m, 5H), 7.10 (s, 1H), 6.48 (s, 1H), 6.46 (d, 2H, J=3 Hz), 6.38 (d, 2H, J=3 Hz), 3.47 (m, 2H), 3.23–3.37 (m, 8H), 3.23 (m, 2H), 1.16–1.19 (m, 12H); ¹³C NMR (100 MHz, CDCl₃, δ ppm): 170.3, 166.9, 153.9, 153.3, 148.9, 134.3, 132.8, 131.1, 130.3, 128.4, 128.1, 127.1, 123.9, 122.8, 108.3, 104.5, 97.7, 65.9, 44.3, 41.9, 39.9, 12.6; ESI-MS: m/z 589.2 [M+H]⁺; Element analysis (%): found: C 75.50, H 6.88, N 9.54, calcd: C 75.48, H 6.85, N 9.52.

**Synthesis of 3**

Isophthalaldehyde (0.166 g, 1.24 mmol) was slowly added into the solution of 1 (1.20 g, 2.48 mmol) in 30 mL MeOH under argon. After the addition three drops of AcOH and some 4 Å molecular sieve, the mixture was refluxed for 8 h. NaBH₄ (0.469 g, 12.4 mmol) was added in three portion at 0 °C. Then the reaction was stirred for 12 h at room temperature before quenching with HCl (5 mL) and adjusted to pH 7 by NaOH aqueous solution. After removal of the solvent, the residue was extracted with HCCl₃ (3 × 15 mL). The combined organic layers were washed with brine and dried over NaSO₄. Removal of the solvents *in vacuo* and purification by silica gel column chromatography with
CH₂Cl₂/MeOH (20/1, v/v) yielded the desired product 3 as solid. yield 85.7%, M.p: 121–123 °C. ¹H NMR (400 MHz, CDCl₃, δ ppm): 7.89 (m, 2H), 7.42 (m, 4H), 7.06 (m, 5H), 6.38 (m, 8H), 6.22 (m, 4H), 3.56 (br, 4H), 3.29–3.35 (m, 20H), 2.45 (br, 4H), 1.14–1.17 (t, 24H, J₆.8 Hz); ¹³C NMR (100 MHz, CDCl₃, δ ppm): 168.7, 153.6, 153.2, 148.7, 139.4, 132.4, 130.9, 128.6, 128.2, 128, 126.8, 123.7, 122.7, 108.1, 105.3, 97.7, 65.1, 52.9, 47.5, 44.3, 39.9, 12.6; ESI-MS: m/z 1071.7 [M+H]+; Element analysis (%): found: C 76.26, H 7.21, N 10.43, calcd: C 76.23, H 7.34, N 10.46.

Synthesis of 4

m-Phthaloyl chloride (0.17 g, 0.843 mmol) was slowly added into the mixture of 1 (0.918 g, 1.897 mmol) and triethylamine (1 ml) in 10 mL CH₂Cl₂ at 0 °C. After the addition, the mixture was stirred at room temperature for 5 h. The solvent was removed and the residue was purified by silica gel column chromatography with EtOAc/PE (2/1, v/v) as eluent to afford 4, yield 95.8%, M.p: 166–168 °C; ¹H NMR (400 MHz, CDCl₃, δ ppm): 8.39 (s, 1H), 8.21 (s, 1H), 8.02 (s, 1H), 8.0 (s, 1H), 7.95 (m, 2H), 7.53 (t, 1H, J=8 Hz), 7.45 (m, 4H), 7.1 (m, 2H), 6.47 (d, 4H, J=8.8 Hz), 6.39 (m, 4H), 6.29 (d, 2H, J=2.4 Hz), 6.27 (d, 2H, J=2.4 Hz), 3.46 (m, 4H), 3.28–3.44 (m, 16H), 3.26 (m, 4H), 1.16 (t, 24H, J=6.8 Hz); ¹³C NMR (100 MHz, CDCl₃, δ ppm): 170.2, 166.4, 153.8, 153.2, 148.9, 134.6, 132.7, 130.4, 129.7, 128.7, 128.4, 128.1, 126.1, 123.8, 123, 108.3, 104.6, 97.7, 65.3, 44.3, 41.5, 40, 12.6; ESI-MS: m/z 1098.6 [M+H]^+; m/z 1121.6 [M+Na]^+; Element analysis (%): found: C 74.26, H 6.76, N 10.21, calcd: C 74.29, H 6.78, N 10.19.

Results and Discussion

Compound 1 was facilely synthesized from rhodamine B and ethylenediamine according to procedures in the literature and obtained as light orange crystals. As shown in Scheme 1, compounds 2 and 4 were prepared in high yield by reacting 1 with benzoyl chloride and m-phthaloyl chloride in the presence of triethylamine respectively. Compound 3 was synthesized in two steps: production of Schiff’s bases from 1 and isophthalaldehyde, followed by reduction by NaBH₄ in MeOH. Their structures have been confirmed using ¹H NMR, ¹³C NMR, ESI mass spectrometry, and elemental analysis (see Supporting Information). Although 4 is a derivative of rhodamine B, it forms a nearly colorless solution in either Tris–HCl aqueous buffer (pH 3–9) or absolute ethanol, indicating that the spirocyclic form exists predominantly. The characteristic peak near 65.0 ppm (9-carbon) in the ¹³C NMR spectrum of 4 also supports this consideration [32]. Besides, neither the color nor the fluorescence (excited at 530 nm) characteristics of rhodamine could be observed for 4 between pH 3.0 and 9.0 in water, suggesting that the spirocyclic form was still preferred in this range (S-Figure 10 and 11). The free probes 2–3 are also colorless and nonfluorescent because they are both in “ring-closed” states. After addition of Fe³⁺ to 2–3 solutions, the complexation induced the three
probes to their “ring-open” states, thus leading to evident color change (from colorless to brilliant pink) and emission of a strong fluorescence. The fluorescence enhancement of 2 to Fe\(^{3+}\), Cr\(^{3+}\) and Fe\(^{2+}\) is as high as 33, 20 and 6-fold, respectively (S-Figure 6). The fluorescence enhancement of 3 (10 \(\mu\)M) to Fe\(^{3+}\) (16 equiv) is as high as 53-fold (S-Figure 8). The colorimetric and fluorometric responses between the probes and Fe\(^{3+}\) can also be conveniently detected by the naked eye (S-Figure 9).

Interestingly, the addition of Fe\(^{3+}\) into the colorless solutions (in both neutral buffer and ethanol) of 4 also generated a purple color and orange fluorescence rapidly, while other ions, such as Li\(^+\), Pb\(^{2+}\), Ag\(^+\), K\(^+\), Co\(^{2+}\), Ni\(^{2+}\), Na\(^+\), Zn\(^{2+}\), Ca\(^{2+}\), Ba\(^{2+}\), Mn\(^{2+}\), Mg\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\) and Hg\(^{2+}\), gave no visible change except for Cr\(^{3+}\) and Fe\(^{2+}\), which caused a mild effect compared to Fe\(^{3+}\) in ethanol-water (1:1). This interesting feature reveals that 4 can serve as a selective “naked-eye” chemosensor for Fe\(^{3+}\) (Fig. 1).

The fluorescence enhancement effects of various metal ions on 4 were investigated under excitation at 548 nm (Figs. 2 and 3). In the absence of metal ions, 4 exhibited a very weak fluorescence peak near 580 nm, which was probably the emission of trace open-ring molecules of 4. When Fe\(^{3+}\) was introduced to a 10 \(\mu\)M solution of 4 in ethanol-water, a bit red shift (~5 nm) and obvious enhancement of fluorescence spectra were observed, whereas other ions of interest displayed much weaker response. In ethanol-water, 4 (10 \(\mu\)M) exhibited a 117-fold enhancement of fluorescence intensity at peak wavelength \(\lambda_{\text{max}} = 575 \text{ nm}\) in the presence of 16 equiv Fe\(^{3+}\). A mild fluorescence enhancement factors (FEF) were also detected for Cr\(^{3+}\) (40-fold), Fe\(^{2+}\) (6-fold), and Li\(^+\), Pb\(^{2+}\), Ag\(^+\), K\(^+\), Co\(^{2+}\), Ni\(^{2+}\), Na\(^+\), Zn\(^{2+}\), Ca\(^{2+}\), Ba\(^{2+}\), Mn\(^{2+}\), Mg\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\) or Hg\(^{2+}\) showed nearly no response (Fig. 2). 4 displays better selectivity and sensitivity than 2 and 3 maybe due to it has more amides to binding the Fe\(^{3+}\).

![Fig. 5](image5.png)

**Fig. 5** Change in the absorbance at 560 nm of 4 (10 \(\mu\)M) in presence of 16 eq of various different metal ions in ethanol–water (1:1, v/v).

![Fig. 6](image6.png)

**Fig. 6** Plots according to the method for continuous variations, indicating the 1:2 stoichiometry for 4-Fe\(^{3+}\) (the total concentration of 4 and Fe\(^{3+}\) is 100 \(\mu\)M). \(X_{\text{Fe}^{3+}} = C_{\text{Fe}^{3+}}/C_{\text{Fe}^{3+}} + C_{4}\)

![Fig. 7](image7.png)

**Fig. 7** Fluorescence intensities of 4 (80 \(\mu\)M) with gradual addition of different amounts of (from bottom 0–8 eq Fe\(^{3+}\)).

![Fig. 8](image8.png)

**Fig. 8** Fluorescence intensity of 4 (10 \(\mu\)M) to Fe\(^{3+}\) in ethanol–water (1:1, v/v) solution. (1) baseline: 10 \(\mu\)M 4 only; (2): 10 \(\mu\)M 4 with 16 eq Fe\(^{3+}\); (3): 4 with 16 eq Fe\(^{3+}\) and then addition of 64 eq PO\(_4^{3-}\) (K salt); (4): 4 with 16 eq Fe\(^{3+}\) and 64 eq PO\(_4^{3-}\), then addition of 16 eq Fe\(^{3+}\); excitation wavelength: 543 nm, slit: 5
Figure 4 shows the absorption spectra of 4 in the presence of various metal ions in ethanol-water. When no metal ion was added to the solution of 4 (10 μM), almost no absorption above 570 nm could be observed, whereas a significant enhancement of the characteristic absorption of rhodamine B emerged soon after Fe3+ was injected into the solution. There was a large enhancement factor (178-fold) of absorbance at 560 nm upon the addition of 16 equiv of Fe3+. A mild increase of absorbance at 560 nm was also detected when the same amount (160 μM) of Cr3+ (causing 61-fold absorption enhancement) was added due to their low binding affinity to 4. Other cations of interest gave no response (Fig. 5).

The Fe3+ binding stoichiometry of 4 can be determined from Fe3+ titration and the Job plot [33]. Detailed kinetic analyses and the mechanisms are discussed later. The “activity Job plots [34, 35]” with a maximum at $X_{Fe}=0.36$ are best fitted to Fe3+:4 = 2:1 (Fig. 6), indicating that the complex (Fe3+)2:4 is the predominant active species.

To further investigate the binding stoichiometry of 4 and Fe3+ ion, a fluorescence titration experiment was carried out. An increase of fluorescence intensity of 4 could be observed with gradual addition of Fe3+ ion. The Fig. 7 indicates that a 1:2 stoichiometry is most possible for the binding mode of Fe3+ and 4 in ethanol-water (1:1). The stability constant ($K_{4}$) of 4 with Fe3+ ion was calculated according to the 1:2 model ($K=2.25 \times 10^5$). The moderate stability constant of the 4-Fe3+ complex is mainly because the need of Fe3+ for six-coordination is not satisfied, and moreover, the strong hydration ability of iron in water. As with many reported rodamine-based spirolactam chemosensors, the Fe3+ induced fluorescence enhancement of chemosensor 4 is most likely the result of the spiro ring-opening mechanism. That is, the chelation of Fe3+ with the oxygen atoms of the amide groups of 4 results in the formation of the open-ring form.

Furthermore, since the color and fluorescence of 4-Fe3+ disappeared immediately when excess EDTA or diethylene-triamine was added, the sensing process was considered to be reversible rather than an ion-catalyzed reaction. Moreover, upon the addition of 64 eq K3PO4 to the solution of 10 μM 4 with Fe3+ (16 eq), the fluorescence intensity at 580 nm was quenched (Fig. 8 blue line) due to the competitive binding of Fe3+ from 4 by K3PO4, and further addition of 40 eq Fe3+ could recover the strong fluorescence again (green line).

Conclusion

In summary, we synthesized a new bis(rhodamine)-based fluorescent probe for Fe3+. This spirolactam compound showed excellent selectivity for Fe3+ in ethanol-water and aqueous Tris–HCl buffer over other common cations, such as Hg2+, Pb2+, Co2+, Ni2+, Zn2+, Cu2+, Cd2+, Ag+, K+ or Li+. The colorimetric and fluorescent response to Fe3+ can be conveniently detected even by the naked eye, which provides a facile method for visual detection of Fe3+. The main limitation of this probe is probably its moderate binding capacity to Fe3+ in aqueous media, which hinders its usefulness in biochemical applications. However, its selectivity is excellent, and the detection of Fe3+ at 10−8 M level is still available. The modification of 4 to develop new fluorescent probes for Fe3+ with stronger binding ability is now under investigation.

Acknowledgments This work was financially supported by the National Science Foundation of China (Nos. 20972143, 20732004 and 20972130)

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