A Highly Selective and Sensitive Turn-On Fluorescent Chemosensor Based on Rhodamine 6G for Iron(III)**

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Introduction

Fluorescent sensors for detection of transition metal ions, such as Cu²⁺, Hg²⁺, and so on, have attracted a great deal of attention in the last decades. Some of the more important among them are selective and sensitive fluorescent sensors for Fe³⁺, where iron, which is a ubiquitous metal in cells, plays vital roles in many biological processes. However, deficiencies or excesses in iron are toxic or can lead to disturbances in glucose levels and lipid metabolism. Though the human body can regulate iron to some extent, detection and analysis of bioactive iron remains an important healthcare challenge for chemists. Most literature reports use fluorescence quenching as the readout mechanism for the sensor response, but very few involve a fluorescence “turn-on” response. Moreover, most turn-on fluorescence sensors for Fe³⁺ are not selective over Cr³⁺ and Cu²⁺. Therefore, new chemosensors that show high selectivity for iron and involve a fluorescence turn-on response appear to be particularly attractive because of the simplicity, high sensitivity, and low detection limit of the fluorescent response.

Recently, more and more rhodamine derivatives have been successfully utilized as fluorophores to construct sensors due to their excellent spectroscopic properties: namely, a large molar extinction coefficient, high fluorescence quantum yield, visible light excitation, and long wavelength emission. As a result, fluorescent chemosensors for Pb²⁺, Hg²⁺, Cr³⁺, Ag⁺, Cu²⁺, and so on, have been developed. Rhodamine derivatives are nonfluorescent and colorless, whereas addition of targeted metal ions leads to ring opening of the corresponding spirolactam, giving rise to a strong fluorescence emission and a color change from colorless to pink. Based on the understanding of the sensing mechanism of rhodamine-based molecular sensors, herein, we report the design and synthesis of a new rhodamine-based chemosensor RAE (Scheme 1), which shows a highly selective and sensitive fluorescence enhancement in response to Fe³⁺ in acetonitrile solution. Moreover, the addition of CN⁻ could quench the fluorescence of the RAE-Fe³⁺ complex, indicating the regeneration of the chemosensor RAE. The color response allows the rapid and accurate recognition of Fe³⁺ with the naked eye, making this new chemosensor a very promising alternative for the detection of Fe³⁺.

Results and Discussion

The chemosensor RAE was synthesized as shown in Scheme 1. Compounds 2[13] and 3[14] were synthesized according to literature methods. The reaction of acetyl chloride 3 and hydroxylamine hydrochloride afforded rhodamine derivative 4 with triethylamine as the base. Subsequently, the chemosensor RAE was obtained through the condensation of 4 and acetyl chloride with a yield of 41%. The structure of RAE was characterized by ¹H NMR and ¹³C NMR spectroscopy. With RAE in hand, we investigated its fluorescence properties by fluorescence measurements. After conducting a preliminary survey with various

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ious solvent systems, we chose acetonitrile for possible application of the system in metal ion analysis.

Upon the addition of different amounts of Fe$^{3+}$, the absorbance intensity of RAE in acetonitrile became enhanced, and a new absorbance peak at ~520 nm was observed (Figure S2 in the Supporting Information). Therefore, we chose 520 nm as the excitation wavelength in the fluorescence experiments. When the colorless solution containing RAE was subjected to fluorescence measurement, the solution (1.0 $\times$ 10$^{-6}$ M) exhibited a very weak emission as shown in Figure 1. At the same time, the pink solution consisting of RAE (1.0 $\times$ 10$^{-6}$ M) and Fe$^{3+}$ (10 equiv) (Figure S3 in the Supporting Information) showed relatively strong fluorescence intensity. Similar to some reported rhodamine-based fluorescent sensors for Fe$^{3+}$,[4f, g] the fluorescence enhancement of RAE solution in the presence of Fe$^{3+}$ is also attributed to the formation of the spirolactam-ring-opened form of rhodamine induced by Fe$^{3+}$. Other tested metal ions did not induce any distinct fluorescence enhancement (Figure 1). Thus, sensor RAE is capable of fluorescence recognition of Fe$^{3+}$ in acetonitrile.

Titration of RAE solution (1 $\mu$M) in acetonitrile by addition of 0–110 equiv Fe$^{3+}$ was subsequently carried out. Upon incremental addition of Fe$^{3+}$, the fluorescence intensity of RAE solution at 548 nm increases gradually and reaches the saturation point when 110 equiv of Fe$^{3+}$ is added (Figure 2). The inset picture shows relative intensity (I/I$_0$) versus the concentration of Fe$^{3+}$ in the low concentration region up to 7.98 ppb.

Achieving a highly selective response to the target analyte over a complex background of potentially competitive species is an important requirement for a chemosensor. Thus, the competition experiments in the presence of potentially competitive metal ions were conducted, and the results are shown in Figure 3. The results of the competitive-metal-ion binding studies clearly suggest a lack of interference by the other metal ions (100 equiv) on the selective detection of Fe$^{3+}$ (10 equiv) by RAE.

We also found that RAE could complex with Fe$^{3+}$ in a 1:1 ratio, confirmed by the Job plot. A maximum emission intensity is seen when the molecular fraction of Fe$^{3+}$ is ~0.50, which indicates the formation of a 1:1 complex between RAE and Fe$^{3+}$ with a total concentration of 10 $\mu$M (Figure 4). The bind-

![Scheme 1. Synthetic route to RAE. Reagents and conditions: a) EtOH, NaOH, H$_2$O, reflux, 2 h, 85 %; b) CH$_3$Cl, POCl$_3$, reflux, 3 h, 91 %; c) CH$_3$Cl, NH$_2$OH-HCl, Et$_3$N, rt, 6 h, 31 %; d) 1, CH$_3$CN, NaH, CH$_3$COCl, 0–5 °C, 30 min, 41 %.

![Figure 1. Fluorescence spectra ($\lambda_{ex}$ = 520 nm) of RAE (1 $\mu$M) in CH$_3$CN with different metal ions (10 equiv) (Other ions = Cu$^{2+}$, Ba$^{2+}$, Ni$^{2+}$, Mg$^{2+}$, Na$^+$, Ca$^{2+}$, Pb$^{2+}$, Ag$^+$, Co$^{2+}$, Zn$^{2+}$, Hg$^{2+}$, Cr$^{3+}$, and Al$^{3+}$).

![Figure 2. Fluorescence titration of RAE (1 $\mu$M) in CH$_3$CN with increasing Fe$^{3+}$ concentration ($\lambda_{em}$ = 520 nm). Inset: the fluorescence of RAE (1 $\mu$M) ($\lambda_{em}$ = 548 nm) as a function of the Fe$^{3+}$ concentration (0–8 $\times$ 10$^{-10}$ M).

![Figure 3. Competitive-metal-ion binding studies (100 equiv) on the selective detection of Fe$^{3+}$ (10 equiv) by RAE.](image-url)
ing ratio of RAE and Fe³⁺ was also confirmed by using the
Benesi–Hidebrand method (Figure 5).

Regeneration of the probe is a prerequisite in developing
novel chemosensors for practical applications. The regenera-
tion of the receptor RAE was performed by the addition of the
Fe³⁺-binding agent CN⁻. As shown in Figure 6, addition of CN⁻ to the solution of receptor RAE and Fe³⁺ results in dimin-
ution of the fluorescence intensity at 548 nm, which indicates
the regeneration of the free receptor RAE. Furthermore, the
fluorescence of the solution of RAE and Fe³⁺ can be recovered
even after four cycles of Fe³⁺ addition followed by CN⁻-in-
duced quenching (Figure 7). Such a regeneration process is im-
portant for the fabrication of Fe³⁺ sensors.

Conclusions

A novel fluorescent sensor RAE was designed and synthesized.
In acetonitrile, RAE exhibits highly selective and sensitive de-
tection of Fe³⁺ over other metal ions with a fluorescence turn-
on effect, and the detection limit is 7.98 ppb. Moreover, the
addition of CN⁻ could quench the fluorescence of the RAE–
Fe³⁺ complex, indicating the regeneration of chemosensor
RAE. Further efforts will be focused on the structure modifica-
tion of the sensor so that it could also be operated in aqueous
solution for possible biological applications.

Experimental Section

Instruments and materials: The fluorescence spectra were record-
ed on a Hitachi F-4500 spectrophotometer. A 1.0 cm quartz cuvette
with a volume of 3.0 mL was used for all spectra collection. Thin-
Figure 7. Regeneration of RAE (1 μM, CH3CN) upon repeated addition of Fe3+ (10 equiv) followed by CN− (25 equiv). λex = 520 nm. Four cycles of Fe3+ and CN− addition are shown. The bars show the fluorescence intensity after addition of Fe3+ (black) and after the addition of CN− (red).

layer chromatography (TLC) was performed on glass plates coated with SiO2 GF254. The plates were inspected by UV light or in I2 vapor. Column chromatography was performed on silica gel (200–300 mesh). ¹H and ¹³C NMR spectra were recorded on a Bruker AV 500 NMR (500 MHz) using tetramethylsilane (TMS) as an internal standard. Matrix-assisted laser desorption/ionization mass spectrometry (MS-MALDI) was performed on a Bruker Daltonics Biflex III. Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified and dried by standard procedures prior to use. CH3CN in chromatographic grade was used throughout the experiments as solvent. For the CH3CN stock solutions as solvent. For the CH3CN stock solutions of the various metal ions (0.01 m), the perchlorate salts of Fe³⁺ (black) and after the addition of CN⁻ to give compound 4 as a pink solid (150 mg, 0.35 mmol, 36% from rhodamine 6G). The pink colored product was recrystallized from CH3Cl2/hexanes (1:1, v/v) to give compound 4 as a white solid (117 mg, 31%). ¹H NMR (400 MHz, CDCl₃): δ = 7.87–7.84 (m, 1H), 7.47–7.42 (m, 2H), 7.07–7.04 (m, 2H), 6.40 (s, 2H), 6.36 (s, 2H), 3.53 (br s, 2H), 3.25–3.20 (m, 2H), 2.13 (s, 6H), 1.24 ppm (t, J = 7.2 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃): δ = 163.6, 152.3, 150.9, 147.7, 132.8, 128.6, 128.3, 127.9, 123.8, 123.0, 117.9, 104.7, 97.1, 65.9, 38.5, 16.9, 14.9 ppm; IR (film): ν = 3390, 2963, 2924, 1683, 1645, 1623, 1519, 1467, 1416, 1377, 1342, 1277, 1208, 1156, 1091, 1044, 1014 cm⁻¹.

3′,6′-Bis(ethylamino)-2-acetoxy-2′,7′-dimethyl-spiro[1H-isindole-1,9′-(9H)xanthen-3(2H)-one] (RAE) To the solution of 4 (1.5 g, 3.73 mmol) in anhydrous CH3CN (45 mL), NaN₄ (0.11 g, 4.5 mmol) was added at 0–5°C. The mixture was stirred in an ice bath for 30 min. CH₃COCl (0.39 g, 4.11 mmol) dissolved in CH₃CN (10 mL) was added at 0–5°C dropwise over 20 min. The mixture was stirred in an ice bath for more than 30 min. Impurities were removed by filtration, and the filtrate was concentrated in vacuum. Purification by flash column chromatography (hexanes/EtOAc, 5:1) gave RAE as an ivory-white solid (0.62 g, 41%); ¹H NMR (500 MHz, CD3COCD3): δ = 10.11 (s, 1H), 7.87 (d, J = 7.0 Hz, 1H), 7.62–7.56 (m, 2H), 7.05 (d, J = 7.5 Hz, 1H), 6.42 (s, 2H), 6.32 (s, 2H), 4.55 (s, 2H), 3.25 (t, J = 6.0 Hz, 2H), 1.95 (s, 9H), 1.29 ppm (q, J = 5.5 Hz, 6H); ¹³C NMR (125 MHz, CD3COCD3): δ = 167.5, 163.4, 153.2, 152.3, 149.1, 134.4, 129.6, 129.0, 124.9, 123.7, 118.9, 105.2, 97.0, 66.6, 39.0, 18.0, 17.3, 14.9 ppm; MS-MALDI: m/z = 472.1 [M + H]+.

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