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A New Rhodamine-Based Chemosensor Exhibiting Selective Fe^{III}-Amplified Fluorescence

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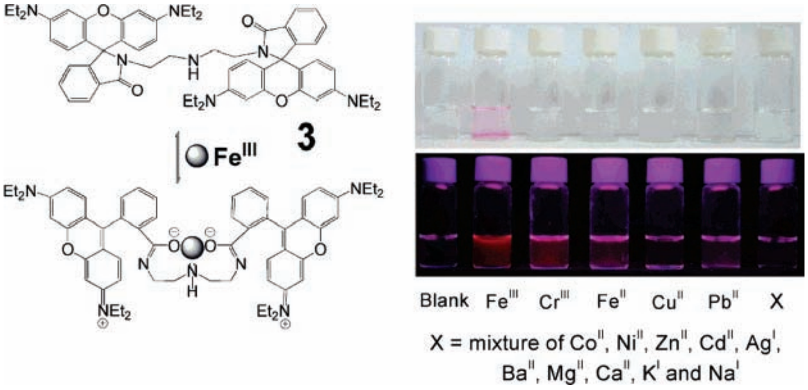
ABSTRACT



A new fluorescent probe 3 was synthesized, and it exhibited high selectivity for Fe^{III} over other commonly coexistent metal ions in both ethanol and water. Upon the addition of Fe^{III}, the spirocyclic ring of 3 was opened and a significant enhancement of visible color and fluorescence in the range of 500–600 nm was observed.

During the recent two decades, there has been a great emergence of interest in the development of fluorescent probes for various cations and anions.[1] Due to their importance in many biological and environmental processes, transition-metal ions have received increasing attention. Numerous excellent works focus on the selective and sensitive detection of transition metal ions; e.g., detection of Cu^{II}, Pb^{II}, Zn^{II}, and Hg^{II} have been reported.[2] Surprisingly, the examples of Fe^{III}-selective fluorescent probes are still scarce[3] despite the indispensable role of Fe^{III} in many biochemical processes at the cellular level.[4] In addition, the ferric ion is well-known as a fluorescence quencher due to its paramagnetic nature, and most of the reported Fe^{III} receptors, such as analogues of ferrichromes or siderophores, undergo a fluorescence quenching when bound with Fe^{III},[5] though it is generally believed that probes with a fluorescence

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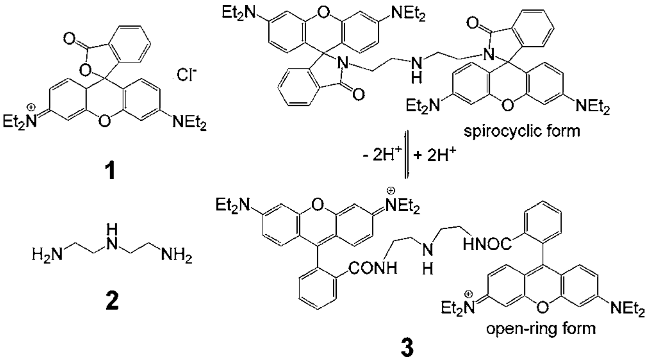

Figure 1. Structrues of ligands **1**−**3**.

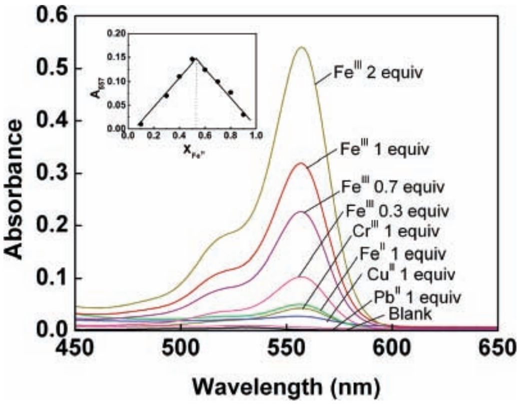

Figure 2. Changes in the absorption spectra of **3** (100 μM) in the presence of different metal ions in absolute ethanol. Inset: plots according to the method for continuous variations, indicating the 1:1 stoichiometry for **3**−Fe^{III} (the total concentration of **3** and Fe^{III} is 100 μM).

enhancement signal when interacting with analytes are much more efficient. Therefore, the development of new fluorescent Fe^{III} indicators, especially those that exhibit selective Fe^{III}-amplified emission, is still a challenge.

On the other hand, rhodamine-based fluorescent chemosensors have received increasing interest in recent years by virtue of their long-wavelength emission and availability.[6] Moreover, it is well-known that many derivatives of rhodamine undergo equilibrium between spirocyclic and ring-open forms, and the two forms always behave with completely different fluorescent properties. In fact, this is an ideal model for the design of light “off-on” switch sensors because the commonly existent spirocyclic forms of these dyes are generally nonfluorescent (“off” signal), and the formation of strongly fluorescent open-ring states will occur (“on” signal) when guests, e.g., protons and metal ions, are bound to the host probes.[6b]

Herein, we report a new rhodamine-based chemosensor **3** (Figure 1), which displayed highly selective Fe^{III}-amplified fluorescence in both ethanol and buffered water.

Compound **3** was facilely synthesized from rhodamine B (**1**) and diethylenetriamine (**2**) and obtained as light orange crystals. Its molecular structure was confirmed by MS, NMR, and element analysis.[7] Although **3** is a derivative of rhodamine B, it forms a nearly colorless solution in either Tris−HCl aqueous buffer (pH = 7.15) or absolute ethanol, indicating that the spirocyclic form exists predominantly. The characteristic peak near 65.0 ppm (9-carbon) in the ^{13}C NMR spectrum of **3** also supports this consideration.[8] Besides, neither the color nor the fluorescence (excited at 510 nm) characteristics of rhodamine could be observed for **3** between pH 5.0 and 9.0 in water, suggesting that the spirocyclic form was still preferred in this range. As the solution became even more acidic (pH < 4.5), however, an obvious enhancement (S-Figure 1, Supporting Information) of color and fluorescence appeared due to the formation of the open-ring state (Figure 1).

Interestingly, the addition of Fe^{III} into the colorless solutions (in both neutral buffer and ethanol) of **3** also generated a purple color and orange fluorescence rapidly, while other ions, such as Co^{II}, Ni^{II}, Zn^{II}, Cd^{II}, Ag^{I}, Pb^{II}, Ba^{II}, Mg^{II}, Ca^{II}, K^{I}, and Na^{I}, gave no visible change except for Cr^{III}, Fe^{II}, and Cu^{II}, which caused a very mild effect compared to Fe^{III} in ethanol but not in water. This interesting feature reveals that **3** can serve as a selective “naked-eye” chemosensor for Fe^{III} (S-Figure 2, Supporting Information).

Figure 2 shows the absorption spectra of **3** in the presence of various metal ions and different amounts of Fe^{III} in absolute ethanol. When no metal ion was added to the solution of **3** (100 μM), almost no absorption above 500 nm could be observed, whereas a significant enhancement of the characteristic absorption of rhodamine B emerged soon after Fe^{III} was injected into the solution. There was a large enhancement factor (154-fold) of absorbance at $\lambda_{\max} = 557$ nm upon the addition of 1 equiv of Fe^{III} (100 μM). A mild increase of absorbance at 557 nm was also detected when the same amount (100 μM) of Cr^{III} (causing 20-fold absorption enhancement), Fe^{II} (24-fold), or Cu^{II} (13-fold) was added due to their low binding affinity to **3**. Other cations of interest gave no response (Figure 2). Similar changes in absorption spectra was also observed when **3** was examined in Tris−HCl aqueous buffer of pH 7.15 (S-Figure 3, Supporting Information).

The fluorescence enhancement effects of various metal ions on **3** were investigated under excitation at $\lambda_{ex} = 510$ nm[9] (Figure 3). In the absence of metal ions, **3** exhibited a very weak fluorescence peak near 550 nm, which was probably the emission of trace open-ring molecules of **3**.

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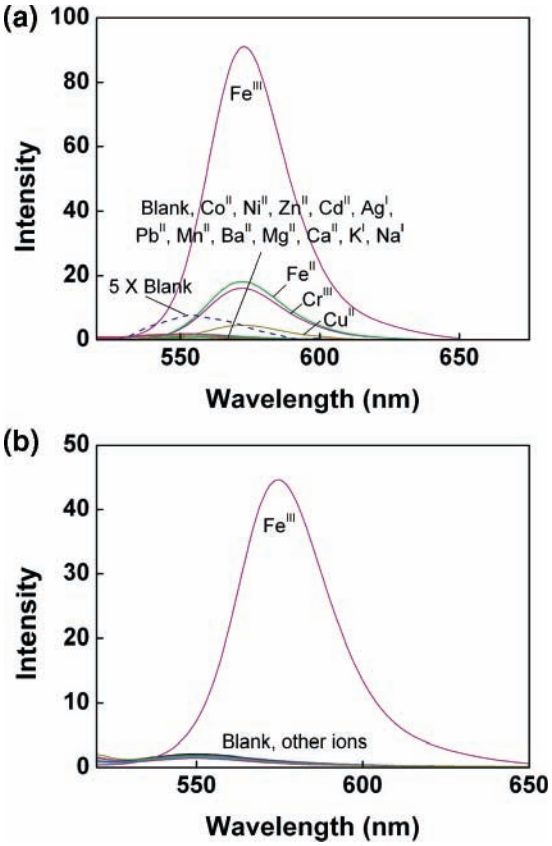

Figure 3. Fluorescence spectra of **3** (10 μM) in the absence and presence of different metal ions in (a) ethanol (20 equiv ions) and (b) Tris-HCl buffer (50 equiv ions, pH = 7.15). For all of the tests, excitation and emission was performed at 510 and 575 nm, respectively.

When Fe^{III} was introduced to a 10 μM solution of **3** in either ethanol or buffered water, obvious red shift (~25 nm) and enhancement of fluorescence spectra were observed, whereas other ions of interest displayed much weaker response. In absolute ethanol, **3** (10 μM) exhibited a 114-fold enhancement of fluorescence intensity at peak wavelength λ_{max} = 573 nm in the presence of 20 equiv Fe^{III}. Very mild fluorescence enhancement factors (FEF) were also detected for Cr^{III} (20-fold), Fe^{II} (23-fold), and Cu^{II} (6-fold), and Co^{II}, Ni^{II}, Zn^{II}, Cd^{II}, Ag^{I}, Pb^{II}, Ba^{II}, Mg^{II}, Ca^{II}, K^{I}, or Na^{I} showed nearly no response (Figure 3a). In Tris−HCl aqueous buffer (pH = 7.15), a lower FEF of 48-fold at λ_{max} = 575 nm was obtained upon the addition of 50 equiv of Fe^{III}. Nevertheless, the selectivity was much higher than that in ethanol, since there was even no fluorescence response of Cr^{III}, Fe^{II}, and Cu^{II} (Figure 3b). The competition experiment was also carried out by adding Fe^{III} to the aqueous solutions of **3** in the presence of other metal ions and showed in Figure 4. The results indicate that the sensing of Fe^{III} by **3** is hardly affected by these commonly coexistent ions.

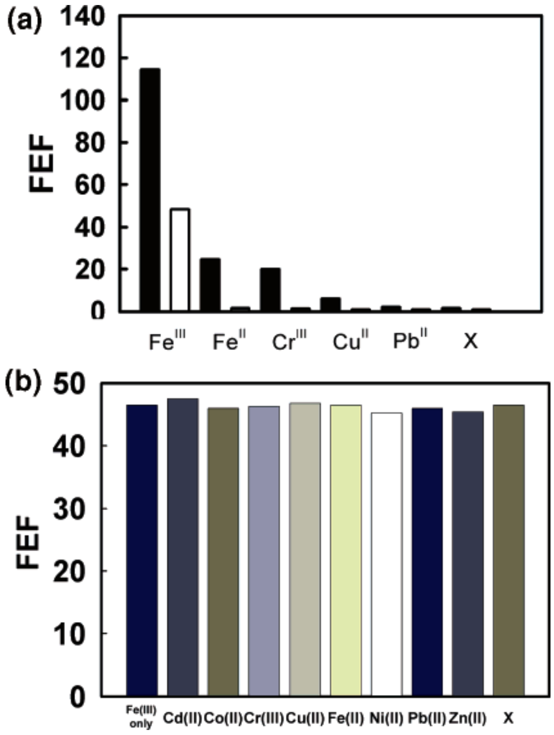

Figure 4. (a) Fluorescence enhancement factors (FEF) of **3** (10 μM) upon the addtion of different metal ions in ethanol (black, 20 equiv of ions) and Tris-HCl buffer (white, 50 equiv ions). X represents Co^{II}, Ni^{II}, Zn^{II}, Cd^{II}, Ag^{I}, Pb^{II}, Ba^{II}, Mg^{II}, Ca^{II}, K^{I}, or Na^{I}. (b) Fluorescence enhancement response of **3** (10 μM in Tris-HCl buffer containing 500 μM Fe^{III}) to 500 μM different metal ions. X is a mixture of Ba^{II}, Mg^{II}, Ca^{II}, K^{I}, and Na^{I}. Excitation and emission was at 510 and 575 nm, respectively.

The fluorescence titration experiments were performed by means of mixing various amounts of metal ions with 30 μM **3** (S-Figure 4a−e, Supporting Information, and Figure 5). It was found that, to generate more than 90% of the total fluorescence enhancement, 15 and 50 equiv of Fe^{III} were necessary in ethanol and Tris−HCl buffer, respectively. The inset of Figure 2 indicates that a 1:1 stoichiometry is most possible for the binding mode of Fe^{III} and **3** in ethanol.[10,11] The stability constant (K) of **3** with different metal ions was calculated according to the 1:1 model (Table 1).[12] The moderate stability constant of the **3**−Fe^{III} complex in aqueous buffer is mainly because the need of Fe^{III} for six-coordination is not satisfied, and moreover, the strong hydration ability of iron in water. However, the detection of Fe^{III} at 10^{-5} M level[3b] with high selectivity is still available using **3** as an indicator.

As with many reported rodamine-based spirolactam chemosensors, the Fe^{III} induced fluorescence enhancement of chemosensor **3** is most likely the result of the spiro ring-

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(9) Excitation was performed at 510 nm rather than 557 nm (the peak of the absorption spectra) to obtain a full view of the fluorescence spectra from 520 to 650 nm.

(10) Vosburgh, W. C.; Cooper, G. R. *J. Am. Chem. Soc.* **1941**, *63*, 437.

(11) The repeatability of the Job's plot analysis in Tris−HCl buffer was poor due to the weak binding capacity of **3** and Fe^{III} in aqueous media and the trend of **3** to precipate in neutral water at high concentration (200 μM).

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Table 1. Stability Constant (*K*) of **3** with Different Metal Ions

metal ions	log *K* in ethanol	log *K* in buffer[a]
Fe^{III}	4.0	3.5
Fe^{II}	2.4	*c*
Cr^{III}	2.3	*c*
Cu^{II}	1.5	*c*
other ions[b]	*c*	*c*

[a] Tris-HCl aqeous buffer (pH = 7.15). [b] Including Co^{II}, Ni^{II}, Zn^{II}, Cd^{II}, Ag^{I}, Pb^{II}, Ba^{II}, Mg^{II}, Ca^{II}, K^{I}, or Na^{I}. [c] Too low to detect.

opening mechanism. That is, the chelation of Fe^{III} with the oxygen atoms of the amide groups of **3** results in the formation of the open-ring form.[6] Furthermore, since the color and fluorescence of **3**$-Fe^{III}$ disappeared immediately when excess EDTA or diethylenetriamine was added, the sensing process was considered to be reversible rather than an ion-catalyzed reaction. The proposed binding mechanism of Fe^{III} with **3** was shown in Figure 5. It should be noted that other coordination sties of the three-coordinate iron may be occupied by solvent oxygens and the counteranions of Fe^{III}. The absence of the 1:2 (Fe^{III}/**3**) binding mode is probably due to the space effect of large rhodamine units in **3**.

In conclusion, we synthesized a new fluorescent probe for Fe^{III} using rhodamine as a fluorophore. This spirolactam compound showed highly selective Fe^{III}-amplified fluorescence emission in both ethanol and aqueous Tris$-$HCl buffer (pH = 7.15). Commonly coexistent metal ions, e.g., Cr^{III}, Fe^{II}, Cu^{II}, Co^{II}, Ni^{II}, Zn^{II}, Cd^{II}, Ag^{I}, Pb^{II}, Ba^{II}, Mg^{II}, Ca^{II}, K^{I}, and Na^{I} displayed little interference, especially when the experiments were operated in buffered water. The enhancement of color and fluorescence in the presence of Fe^{III} was in the range of 500~650 nm; thus, the compound was able to serve as a "naked-eye" chemosenor for ferric ion. The main limitation of this probe is probably its moderate binding capacity to Fe^{III} in aqueous media, which hinders its usefulness in biochemical applications. However, its selectivity is excellent, and the detection of Fe^{III} at 10^{-5} M level is still available. The modification of **3** to develop new fluorescent probes for Fe^{III} with stronger binding ability (e.g., the proper connection of **3** with other receptor molecules to yield a six-coordinate probe) is now under investigation.

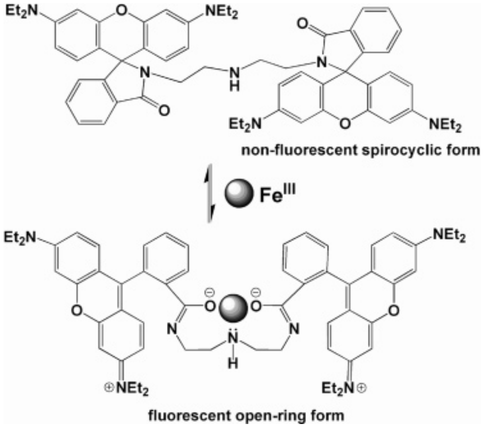

Figure 5. Proposed mechanism for the fluorescence enhancement of **3** upon the addtion of Fe^{III}. The solvent oxygens and counter-anions were omitted for clarity.

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Supporting Information Available: Experimental procedures, characterization data for the compounds described, and selected spectroscopic data of **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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