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Rhodamine-hydroxamate-based fluorescent chemosensor for Fe^{III}

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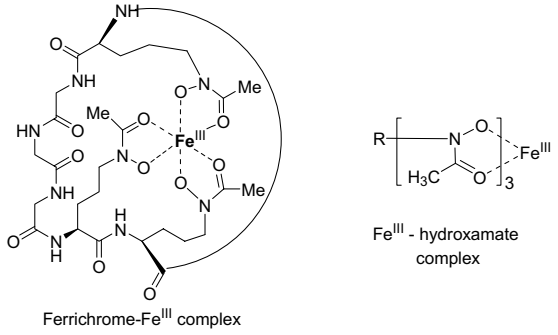
Abstract—A hydroxamate-based chemosensor, which responds fluorescently and colorimetrically to Fe^{III} in micromolar ranges, has been developed. The rhodamine hydroxamate probe is prepared in two steps from rhodamine B base. The biomimetic hydroxamate binding site is attached to the rhodamine fluorophore to recognize Fe^{III} selectively over other biologically important metal ions.

Selective fluorescent chemosensors are especially important for the monitoring of biologically essential metal ions. An enormous effort has gone into the development of selective fluorescent chemosensors[1] due to their high sensitivity and easy applicability for in vitro and in vivo applications. Assessing of biologically relevant metal ions, such as Ca^{II},[2] Zn^{II},[3] Cu^{I},[4] Mg^{II},[5] Hg^{II},[6] and Pb^{II} in cells[7] has been successfully applied.

Iron is one of the most essential metals in the biological systems and plays crucial roles in cellular metabolisms.[8] Especially, ferric iron (Fe^{III}) is widely retained in many proteins and enzymes either for structural purposes or as part of a catalytic site.[9] However, selective and sensitive fluorescent chemosensors for Fe^{III} are still rare. Most of the known Fe^{III} sensors are based on the fluorescence quenching mechanisms due to the paramagnetic nature of ferric ions.[10] Recently, few examples employing fluorescent enhancements upon Fe^{III} bindings have been reported.[11]

Biological iron^{III} uptake processes utilize siderophores, such as analogues of ferrichrome or enterobactin,[12] which possess either hydroxamates or catecholates as binding sites. The structures of ferrichrome–Fe^{III} and genralized hydroxamate–Fe^{III} complexes are shown in Scheme 1. Fluorescently-labeled ferrichrome analogues having the hydroxamate binding sites have been developed for monitoring of iron^{III} uptakes,[13] where fluorescence is quenched upon iron binding. Herein, we report a new turn-on fluorescent chemosensor for Fe^{III} based on the biomimetic hydroxamate binding unit coupled with the rhodamine fluorophore.



Scheme 1. Structures of ferrichrome and the generalized 3:1 hydroxamate/Fe^{III} complex found in siderophores.

By introducing the hydroxamate unit into the rhodamine amide's equilibrium[14,11a,b] between spirolactam to ring opened amide, we designed rhodamine hydroxamate **1**[15] as a new Fe^{III} probe. Compound **1** is prepared from rhodamine B base by using a two step procedure ((1) $POCl_3$, $ClCH_2CH_2Cl$, reflux, 4 h; (2) $MeONH_2{\cdot}HCl$, Et_3N, CH_2Cl_2, rt, 4 h)[16] in 81% yield for two steps (Scheme 2). This substance forms a colorless solution in MeOH–MeCN (1:1 v/v) and shows no significant fluorescence signals.[17] However, addition of Fe^{III} into the solution induces a red-purple color and strong fluorescence rapidly.

Fluorescence titration of **1** (20 μM) with Fe^{III} was performed by using a MeOH–CH_3CN (1:1 v/v)[18] solution at 25 °C. Upon addition of Fe^{III}, the fluorescence intensity of the solution increases gradually (Fig. 1a) and

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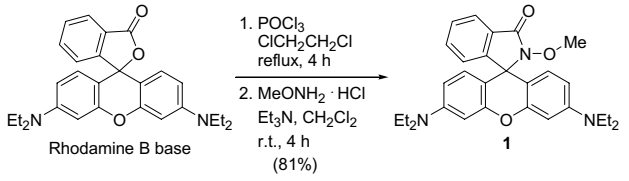


Scheme 2. Synthesis of rhodamine hydroxamate **1**.

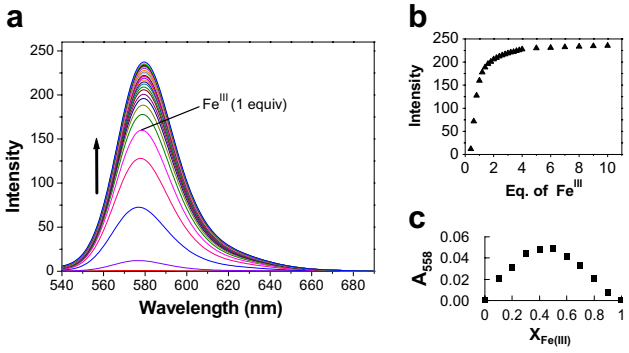
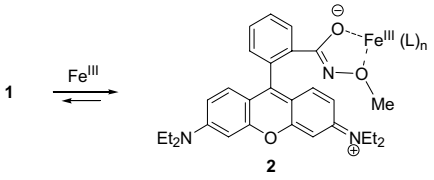


Figure 1. (a) Fluorescence emission of a solution of **1** (20 μM) in MeOH–CH_3CN (1:1 v/v) upon addition of Fe^{III} (excitation at 530 nm). (b) Plot of fluorescence intensity at 580 nm versus number of equivalents of Fe^{III}. (c) Job's plot according to the method of continuous variations, indicating the 1:1 stoichiometry of **1**–Fe^{III}.

saturation behavior is observed after addition of 4 equiv of Fe^{III} (Fig. 1b). Although the natural tripodal siderophores utilize all three hydroxamate binding units to complex one ferric ion, we could not observe 3:1 complexation of **1** and Fe^{III}. Job's plot according to the method for continuous variations[19] indicates 1:1 binding[20] stoichiometry (Fig. 1c). The calculated binding constant ($\log K = 5.2$) in MeOH–CH_3CN (1:1 v/v) solution from the UV-titration experiment based on the 1:1 binding model[21] shows a strong binding ability of **1** to Fe^{III}.

The reversible binding mode of **1** and Fe^{III} described in Scheme 3 is demonstrated by observing immediate disappearance of fluorescence (and color) upon the addition of excess of EDTA into the solution of **1**/Fe^{III}.

The fluorescence responses of chemosensor **1** by other metal ions, such as Hg^{II}, Fe^{II}, Cu^{II}, Co^{II}, Zn^{II}, Ag^{I}, Cd^{II}, Pb^{II}, Ba^{II}, Mg^{II}, Ca^{II}, Ni^{II}, Mn^{II}, Na^{I}, and K^{I}, were conducted in order to evaluate the selectivity. Fluorescence intensity changes of **1** (20 μM) in the presence of 4 equiv of each of these metal ions were analyzed in MeOH–



Scheme 3. Proposed 1:1 binding mode of **1** with Fe^{III}.

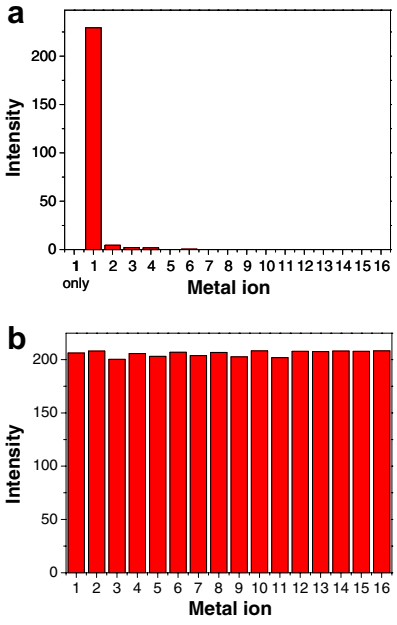


Figure 2. Fluorescence intensities of **1** (20 μM) in MeOH–CH_3CN (1:1 v/v) at 580 nm: (a) in the presence of 4 equiv of the following metal ions: (1) Fe^{III}; (2) Hg^{II}; (3) Fe^{II}; (4) Cu^{II}; (5) Co^{II}; (6) Zn^{II}; (7) Ag^{I}; (8) Cd^{II}; (9) Pb^{II}; (10) Ba^{II}; (11) Mg^{II}; (12) Ca^{II}; (13) Ni^{II}; (14) Mn^{II}; (15) Na^{I}; (16) K^{I}; (b) in the presence of 4 equiv of Fe^{III} and 4 equiv the following metal ions: (1) none; (2) Hg^{II}; (3) Fe^{II}; (4) Cu^{II}; (5) Co^{II}; (6) Zn^{II}; (7) Ag^{I}; (8) Cd^{II}; (9) Pb^{II}; (10) Ba^{II}; (11) Mg^{II}; (12) Ca^{II}; (13) Ni^{II}; (14) Mn^{II}; (15) Na^{I}; (16) K^{I}.

CH_3CN (1:1 v/v). Figure 2a demonstrates a highly selective response of **1** to Fe^{III}. Other metal ions than Fe^{III} do not cause any significant fluorescence intensity changes under the same conditions. And the fluorescence intensity changes caused by the addition of Fe^{III} are not influenced by the presence of other metal ions as shown in Figure 2b.

The fluorescent selectivity of chemosensor **1** for Fe^{III} is matched when **1** is employed as a colorimetric detector. While the reaction of **1** (20 μM) with Fe^{III} results in red-purple color change, no significant color changes are promoted by the addition of other metal ions (Fig. 3).

In summary, we have developed a highly selective and sensitive chemosensor for detecting micromolar concentrations of Fe^{III}. Rhodamine hydroxamate **1** that has a biomimetic hydroxamate binding unit is easily prepared in two steps from rhodamine B base.[22] This chemosensor binds with Fe^{III} in a 1:1 stoichiometric manner to induce a large increment in the fluorescence intensity

Figure 3. Color changes of **1** (20 μM) in MeOH–CH_3CN (1:1 v/v) in the presence of 2 equiv of metal ions.

and a marked color change. Importantly, the selectivity of this system for Fe^{III} over other metal ions is extremely high.

Acknowledgements

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Supplementary data

Experimental procedures for the synthesis, spectral data, and copies of ^{1}H NMR and ^{13}C NMR of **1**, data for UV–vis, fluorescence of **1**, and other data are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.06.014.

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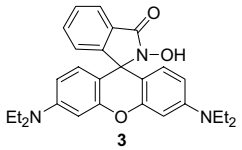
15. According to the initial screening of rhodamine hydroxamate derivatives, hydroxamic acid **3** shows strong bindings toward Cu^{II}, Hg^{II}, and Fe^{III}. See Supplementary data (p 10).



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17. This indicates that compound **1** exists predominantly in the spirocyclic form.
18. In a pure acetonitrile or methanol solution,**1** binds to Hg^{II} in addition to Fe^{III}. See Supplementary data (p 11).
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20. The reason for the 1:1 binding (not 3:1) between **1** and Fe^{III} is probably due to the unfavorable steric congestion associated if three bulky rhodamine binding units bind one Fe^{III}. See Ref. 11b for a similar steric effect.
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22. *Spectral data for* **1**: mp 98–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.93$–7.91 (m, 1H), 7.48–7.41 (m, 2H), 7.07–7.05 (m, 1H), 6.57 (s, 1H), 6.55 (s, 1H), 6.41 (d, $J = 2.6$ Hz, 2H), 6.31–6.30 (d, $J = 2.6$ Hz, 1H), 6.29–6.28 (d, $J = 2.6$ Hz, 1H), 3.14 (s, 3H), 3.36–3.30 (q, $J = 7.0$ Hz, 8H), 1.17–1.14 (t, $J = 7.0$ Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.9, 153.8, 150.6, 149.0, 133.0, 129.2, 128.8, 128.4, 123.9, 123.1, 108.1, 105.1, 98.0, 65.1, 65.0, 44.5, 12.8; IR (film, cm^{-1}) 3366, 2971, 2929, 2896, 1711, 1615, 1515, 1466, 1426, 1328, 1220, 1119, 1080; HRMS (FAB) *m/z* Calcd for $C_{29}H_{34}N_3O_3$ (M^+) 472.2600, found: 472.2601.