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Fe^{3+}-selective fluorescent probe based on rhodamine B and its application in bioimaging

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ABSTRACT

A Rhodamine-based fluorescent and colorimetric chemosensor for Fe^{3+} ion, acetyl rhodamine-hydroxamate (**ARH**), was designed and synthesized. Upon mixed with Fe^{3+} in CH_3CN–H_2O (1:1, v/v), the spirolactam of **ARH** was opened, which resulted in the dramatic enhancement of both fluorescence and absorbance intensity as well as the color change of the solution. Background metal ions showed small or no interference with the detection of Fe^{3+}. The Job's plot indicated the formation of 1:1 complex between **ARH** and Fe^{3+}. Confocal laser scanning microscopy experiments showed that **ARH** could be used to detect Fe^{3+} in living cells.

1. Introduction

As an essential element for life, Fe^{3+} plays an important role in many chemical and biological processes. Both its deficiency and overdose can induce a wide variety of diseases [1–3]. Therefore, the development of analytical methods for the sensitive and selective determination of Fe^{3+}, is highly desirable. Because of its operational simplicity, low cost, real time monitoring and high selectivity, fluorescent detection has become the promising strategy used for Fe^{3+} detection [4,5]. Recently, some fluorescent chemosensors for ferric have been reported [6–16]. However, most of them have disadvantage in detecting Fe^{3+}, such as tardy sensitive, interference from other metal ions (especial Cu^{2+}), mild fluorescence enhancement or quenching and no obvious color changes. Moreover, many Fe^{3+}-selective fluorescent probes are hydrophobic, and this incompatibility with aqueous environments restricts the applications of these sensors in biological system. Therefore, developments of "turn-on" fluorescent chemosensors that can selectively detect Fe^{3+} in aqueous media with color changes are significant.

The rhodamine framework is an ideal mode to construct fluorescent chemosensors due to its excellent photophysical properties such as long absorption and emission wavelength, large absorption coefficient and high fluorescence quantum yield [17]. Many rhodamine derivatives have been used as fluorescent probes for metal ions, such as Cu^{2+}, Pb^{2+} and Hg^{2+} [18–28]. Although some rhodamine based probes for Fe^{3+} have been reported [29–34], however, only few of them have been applied in biological systems for the detection of Fe^{3+} [35]. Herein, we report a new turn-on fluorescent chemosensor based on acetyl rhodamine-hydroxamate (**ARH**), which can sensitively and selectively detect Fe^{3+} in aqueous media and display enhanced fluorescence intensities and clear color changes upon recognition. Moreover, **ARH** can be applied in biological systems for the detection of Fe^{3+} through confocal laser scanning microscopy experiments.

2. Experimental

All cations in the form of perchloride salts were purchased from Sigma–Aldrich Chemical Company and used without further purification. All other chemicals used were local products of analytical grade. All solvents used in spectroscopic test are spectroscopic grade. Rhodamine B hydroxyamide (**1**) was synthesized according to the literature [36]. A Hitachi F-2500 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 DMX-500 spectrometer at 500 MHz in CD_3COCD_3. Laser confocal scanning microscopy (FluoView FV1000, Olympus) was used for detecting **ARH**–Fe^{3+} in cells. Elemental analyses were carried out with a Flash EA 1112 instrument.

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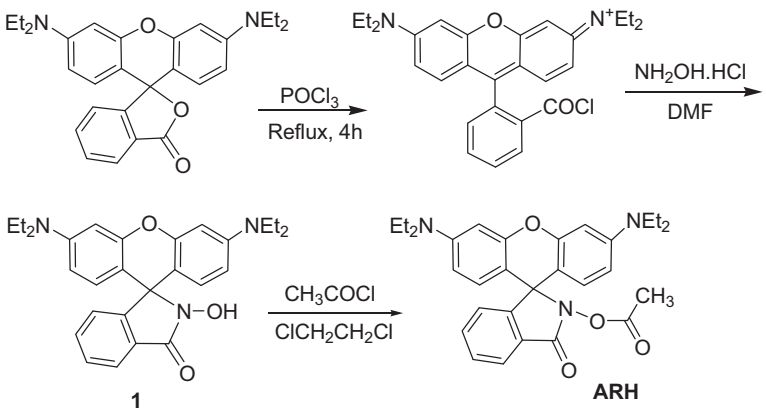

Scheme 1. Synthesis of **ARH**.

2.1. Synthesis

Acetyl chloride (0.24 g, 3 mmol) was slowly added into the mixture of **1** (0.457 g, 1 mmol) and triethylamine(0.303 g, 3 mmol) in 10 mL $ClCH_2CH_2Cl$ at 0 °C. After the addition, the mixture was stirred at room temperature for 4 h. The solvent was removed and the residue was purified by silica gel column chromatography with EtOAc/cyclohexane (1/3, v/v) as eluent to afford 0.22 g **ARH** (yield 44%), m.p. 229–231 °C. ^{1}H NMR (500 MHz, CD_3COCD_3): δ 7.87(d, J = 7.5 Hz, 1H), 7.86–7.60(m, 2H), 7.08(d, J = 7.5 Hz, 1H), 6.62(d, J = 8.5 Hz, 2H), 6.42–6.39(m, 4H), 3.38(q, J = 7 Hz, 8H), 1.95(s,3H), 1.14(t, J = 7.0 Hz, 12H). ^{13}C NMR (250 MHz, CD_3COCD_3): 205.3, 166.6, 162.6, 153.7, 151.2, 149.1, 133.5, 128.9, 128.7, 127.8, 123.9, 122.8, 107.9, 104.3, 97.5; 65.4, 44.0, 17.0, 12.0; ESI–MS 500.3 $[M+H]^+$; Calcd (%) for $C_{30}H_{33}N_3O_4$: C 72.12, H 6.66, N 8.41. Found: C 72.33, H 6.43, N 8.32.

2.2. General procedure for metal ions detection

A solution of **ARH** (1 mM) was prepared by dissolving the requisite amount of **ARH** in CH_3CN, which was diluted with CH_3CN to prepare the stock solution of **ARH** (1×10^{-5} mol L^{-1}). Stock solutions of various other ions were prepared by dissolving their salts in water.

To 10-mL glass tubes containing different amounts of metal ions, proper amounts of the solution of **ARH** was added directly with micropipette, then diluted with H_2O and CH_3CN to 10 mL and mixed. The absorption and fluorescence sensing of metal ions were measured immediately.

3. Results and analysis

3.1. Synthesis

The synthesis of **ARH** is shown in Scheme 1, which can be prepared by the reaction of rhodamine B hydroxyamide (**1**) and acetyl chloride in the prescnece of triethyl amine. The structure of **ARH** was confirmed by ^{1}H NMR, ^{13}C NMR, MS and EA.

3.2. UV–vis spectral responses of *ARH*

All measurements of spectra were carried out in a CH_3CN–H_2O (1:1, v/v) solution. As shown in Fig. 1, UV–vis spectrum of **ARH** ([**ARH**] = 1.0×10^{-5} M) exhibited only very weak bands over 500 nm. Addition of 1 equiv Fe^{3+} into solution immediately resulted in a significant enhancement of absorbance at about 563 nm and a shoulder peak appeared at 525 nm as well as color change of solution from colorless to red. Under the identical condition, no obvious response could be observed upon the addition of other ions including Zn^{2+}, Mg^{2+}, Ca^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Hg^{2+}, Ba^{2+}, Ni^{2+}, Ce^{3+}, K^+, Ag^+, Co^{2+} and Na^+. The results demonstrated that **ARH** was characteristic of high selectivity toward Fe^{3+} over other competitive metal ions.

3.3. Fluorescence spectral responses of *ARH*

ARH([**ARH**] = 1.0×10^{-6} M) in CH_3CN–H_2O (1:1, v/v) solution shows a very weak fluorescence in the absence of metal ions. However, the addition of Fe^{3+} ion resulted in a remarkably enhanced fluorescence at 575 nm. The color of the solution also changed from colorless to pink-red. This strongly suggested that **ARH** can serve as a "naked eye" probe for Fe^{3+}. Under the same conditions, additions of other metal ions including Zn^{2+}, Mg^{2+}, Ca^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Ba^{2+}, K^+, Hg^{2+}, Ag^+, Ni^{2+}, Ce^{3+}, $Co^{2+}$and Na^+ created no obvious fluorescent enhancements and color changes (Fig. 2). These observations further indicated that **ARH** had a high sensitivity and excellent selectivity for Fe^{3+} ion in aqueous media.

To further investigate the interaction of **ARH** and Fe^{3+} ion, a fluorescence titration experiment was carried out. An increase of fluorescence intensity of **ARH** could be observed with gradual addition of Fe^{3+} ion (Fig. 3). Binding analysis using the method of continuous variations (Job's plot) established that the stoichiometry of the **ARH**–Fe^{3+} complex was estimated to be 1:1 (Fig. 4). The

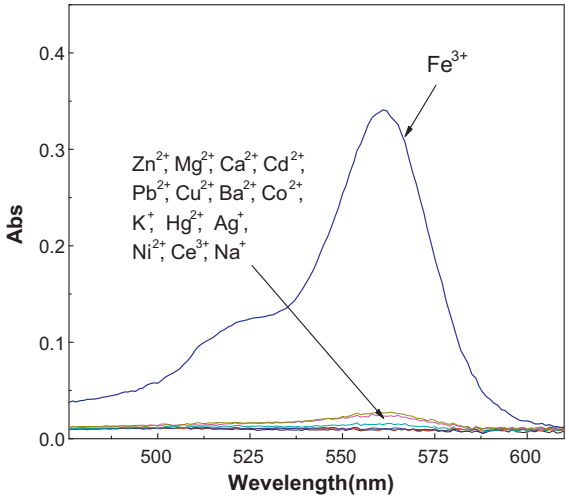

Fig. 1. UV–vis absorption of **ARH** (10 μM) in CH_3CN–H_2O (1:1, v/v) with 10 μM metal ions (Fe^{3+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Ba^{2+}, K^+, Hg^{2+}, Ag^+, Ni^{2+}, Ce^{3+}, Co^{2+}, Na^+).