A new rhodamine fluorescent OFF-ON-OFF probe for selective sensing of Fe$^{3+}$ and AcO$^{-}$ in aqueous media and living cells

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Abstract. A new fluorescence chemosensor for Fe$^{3+}$ was developed based on a rhodamine platform. L displayed highly selective and sensitive “OFF-ON” fluorescence response and naked-eye color change to Fe$^{3+}$ in aqueous solution. The resulting L$^{-}$Fe$^{3+}$ complex was found to act as a selective “ON-OFF” fluorescence probe for AcO$^{-}$ against common anions and cations with a Fe$^{3+}$ displacement approach. The detection limits of L to Fe$^{3+}$ and L$^{-}$Fe$^{3+}$ complex to AcO$^{-}$ were estimated to be 6.0×10$^{-8}$ mol/L and 7.5×10$^{-8}$ mol/L, respectively. The good biocompatibility of L enabled the investigation of fluorescent response for Fe$^{3+}$ and AcO$^{-}$ in living Ana-1 cells by confocal microscope.

1. Introduction

As an essential element for life, iron plays an important role in a wide range of biochemical processes [1], several biological functions depend directly or indirectly on the proper concentration and oxidation states of iron to maintain the homeostatic mechanism of biosystem[2,3]. Its deficiency and excess can induce a variety of diseases [4, 5]. Therefore, it is highly desirable to develop fluorescent probes for the real-time monitoring of iron ions in environmental and living systems [6, 7]. Due to great sensitivity, fluorescent chemosensors combining ionophores and fluorophores are commonly considered superior [8–10]. In recent years, rhodamine has been attracted a great deal of attention due to its excellent properties, including good photostability, long emission wavelength, high fluorescence quantum and so on[11–13]. However, for the majority of rhodamine fluorescent probes, their anti-interference ability is poor, which are usually chelate with two or more metal ions simultaneously[14]. Due to the interference of Cr$^{3+}$, Al$^{3+}$, Cu$^{2+}$ in the identification process of Fe$^{3+}$[15–18], rhodamine fluorescent probe exhibited low selectivity for Fe$^{3+}$[19,20]. The recognition and sensing of anions by proper design of anion receptors is currently of major interest because anions play a fundamental role in chemical and biological processes. Various kinds of anions such as F$^{-}$, Cl$^{-}$, I$^{-}$, PO$_4^{3-}$, CH$_3$COO$^{-}$, etc. play a major role both in environmental and biological systems [21–23]. Among the above anions, AcO$^{-}$ received the most attention from chemists because of its unique properties, the detection of AcO$^{-}$ with the help of easily synthesized receptor and minimal instrumental assistance is desirable towards practical applications [24, 25]. Recently, rhodamine-cation complex solution has been documented for the recognition of various anions [26–28]. This intrigued us to design a novel Fe$^{3+}$-fluorophore complex to develop a AcO$^{-}$-specific fluorescence chemosensor.

In this work, the allyl group was introduced into p-Hydroxybenzaldehyde to improve the charge distribution of probe, herein we designed and synthesized a new turn-on fluorescence sensor based on rhodamine for the quantification of Fe$^{3+}$. The in situ formed L$^{-}$-Fe$^{3+}$ complex solutions exhibited a sensitive response to AcO$^{-}$ via a turn-off response by the displacement strategy. The reversible “off-on-off” fluorescence sensor in solution showed the good application in live Ana-1 cell imaging.

2. Results and discussion

The synthetic route for compound L was shown in Scheme 1. The structure of L was confirmed by NMR, mass spectra (Fig.S1–S5). The detailed experimental procedures and the characterization of the compound L were described in the experimental section. The recognition profiles of compound L were investigated in CH$_3$CN/H$_2$O solutions by UV-vis spectroscopy toward various heavy metal and transition
metals, including Ca\(^{2+}\), Cd\(^{2+}\), Cu\(^{2+}\), Hg\(^{2+}\), Mn\(^{2+}\), Ni\(^{2+}\), Pb\(^{2+}\), Zn\(^{2+}\), Fe\(^{3+}\), Na\(^{+}\), Al\(^{3+}\), Mg\(^{2+}\), Ag\(^{+}\), Cr\(^{3+}\). As presented in Fig. 1, free probe L showed an absorption band at 558 nm, when 10 equiv. of Fe\(^{3+}\) was added to the solutions of probe L, the absorption intensity was significantly enhanced, the change in spectrooscope was come along obvious color change from colorless to pink. Differently, nearly no significant adsorption changes could be observed in the presence of other interested metal ions. These results clearly suggested that L can serve as a “naked-eye” Fe\(^{3+}\) indicator in aqueous media.

Fig. 1 Absorbance spectra of L (10μM) in the absence and presence of 10 equiv. different metal ions in CH\(_3\)CN/H\(_2\)O (v/v=3:7, 10mM Tris-HCl buffer, pH =7.2) solution.

In addition, we have tested the fluorescence properties of L toward a series of metal ions such as Cu\(^{2+}\), Pb\(^{2+}\), Fe\(^{3+}\), Fe\(^{2+}\), Hg\(^{2+}\), Al\(^{3+}\), Cr\(^{3+}\), Ni\(^{2+}\), Mn\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Ag\(^{+}\), Ba\(^{2+}\), Cd\(^{2+}\) and Na\(^{+}\) (100μM) in CH\(_3\)CN/H\(_2\)O buffer (3:7, v/v, 20mM, pH=7.2). As shown in Fig. 2, fluorescence probe L alone and other metal ions all displayed weak fluorescence emission bands except for Fe\(^{3+}\), the addition of Fe\(^{3+}\) induced a remarkable fluorescence enhancement (59-fold), whereas the addition of other metal ions to the solution L exhibited no obvious change in the fluorescence spectra. Meanwhile, the emission color of solutions can be clearly observed to change from colorless to red-orange under illumination with 365 nm light in presence of Fe\(^{3+}\), indicating that there were some interaction between L and Fe\(^{3+}\). The specific response of L towards Fe\(^{3+}\) should be attributed to a bonding change opening the function of the spirolactam ring [29-31]. Compound L can be considered as a new off-on fluorescent chemosensor for Fe\(^{3+}\).

The interference of the potentially competing metal ions toward Fe\(^{3+}\) detection was also investigated. As shown in Fig. 3, chemosensor L was treated with 10 equiv. of Fe\(^{3+}\) and 10 equiv. of other coexistent metal ions such as Cu\(^{2+}\), Pb\(^{2+}\), Fe\(^{3+}\), Hg\(^{2+}\), Al\(^{3+}\), Cr\(^{3+}\), Ni\(^{2+}\), Mn\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Ag\(^{+}\), Ba\(^{2+}\), Cd\(^{2+}\) and Na\(^{+}\). As a result, no interference was observed for the detection of Fe\(^{3+}\) by L, these results manifest the recognition of Fe\(^{3+}\) was not interfered by these competing metal ions.

Fluorescence titration of L (10μM) with Fe\(^{3+}\) was performed in CH\(_3\)CN/H\(_2\)O (v/v=3:7) solution. As shown in Fig.5, the fluorescence intensity increased gradually in the presence of increasing amounts of Fe\(^{3+}\) (0-10 equiv.). Job plot was utilized to determine the binding stoichiometry of the L-Fe\(^{3+}\) complex, it revealed that L and Fe\(^{3+}\) formed a complex in 1:1 stoichiometry (Fig.4) [32]. Hildebrand-Bebesiplots gave binding constants of 2.9x10\(^4\) M\(^{-1}\) in HEPES buffer [33] (Fig. 5). The detection limit for Fe\(^{3+}\) was estimated to be 6.04x10\(^{-4}\)M based on a 3σ/slope analysis (Fig. 6) [34]. Which was sufficiently low to detect submicromolar concentration of the Fe\(^{3+}\), implying that L had great potential for use in the development of Fe\(^{3+}\) sensor materials.

Fig. 2 Fluorescence spectra of L (10μM) upon addition of 10 equiv. of various metal ions in CH\(_3\)CN/H\(_2\)O (v/v=3:7, 10mM Tris-HCl buffer, pH=7.2) solution, λ\(_{ex}\)= 520nm.

Fig. 3 Fluorescence responses of L (10μM) to various metal ions (10 equiv.) in CH\(_3\)CN/H\(_2\)O (v/v=3:7, 10mM Tris-HCl buffer, pH=7.2) solution. Bars represent the final integrated fluorescence response (F) over the initial integrated emission (F0). Green bars represent the addition of an excess of the appropriate metal ion (10 equiv.) to a 10μM solution of L. Red bars represent the addition of Fe\(^{3+}\) (10 equiv.) to the solution (λ\(_{ex}\)=520nm). (1)Fe\(^{3+}\); (2)Cu\(^{2+}\); (3)Pb\(^{2+}\); (4)Fe\(^{2+}\); (5)Hg\(^{2+}\); (6)Al\(^{3+}\); (7)Cl\(^{-}\); (8)Ni\(^{2+}\); (9)Mn\(^{2+}\); (10)Co\(^{2+}\); (11)Zn\(^{2+}\); (12)Ag\(^{+}\); (13)Ba\(^{2+}\); (14)Cd\(^{2+}\); (15)Na\(^{+}\).

Fig. 4 Job’s plot evaluated from the fluorescence spectra of L and Fe\(^{3+}\) in buffered aqueous (λ\(_{ex}\)=520nm)
The fluorescence intensity of L gradually decreased with the addition of increasing concentration of AcO (0-20 μM) in Tris-HCl aqueous buffer (CH₃CN/H₂O (v/v=3:7), 10mM, pH=7.2) solution (λex=520 nm). The detection limit of L-Fe³⁺ and AcO⁻ was determined to be 7.51×10⁻³ mol/L (Fig. S8).

Fig. 8 Fluorescence responses of L (10 μM) to Fe³⁺ and L-Fe³⁺ complex to AcO⁻ in CH₃CN/H₂O (v/v=3:7, 10mM Tris-HCl buffer, pH=7.2) solution (λex=520 nm).

The effect of pH on the fluorescence of probe L to Fe³⁺, L-Fe³⁺ complex to AcO⁻ was evaluated. As shown in Fig. 9, L and L-Fe³⁺ were all weakly fluorescent over a wide pH range (pH=4.0-10.0). Meanwhile, probe L displayed an efficient fluorescence response to Fe³⁺ in the pH range of 5.0-8.0. It can be seen that the fluorescence intensity of L-Fe³⁺ is evidently enhanced (pH≥5) and reaches its maximum at pH 7. The results indicated that the L may be used as a chemosensor for Fe³⁺ detection.

Fig. 9 Effect of pH to the react of probe L (10 μM) to Fe³⁺ (10 equiv.) and L-Fe³⁺ complex to AcO⁻ (10 equiv.) in CH₃CN/H₂O (v/v=3:7) solution.

The proposed displacement strategy for the detection of AcO⁻ was further supported by the results of mass spectra studies, FT-MS spectra of L showed a molecular ion peak [L+H]+ at m/z 601.3146 (Fig. S3). When the Fe³⁺ ion was added into the solution of L, the peak at m/z 718.4273 is assignable to [L-2H⁺+Fe³⁺+NO₃⁻+H⁺]⁺ species (Fig. S4). This result confirmed that 1:1 stoichiometry complex between L and Fe³⁺. In the presence of AcO⁻, FT-MS spectra displayed a molecular ion peak at m/z 601.31695 again (Fig. S5), indicating that the decomplexation of Fe³⁺ ion in the presence of AcO⁻ led to the liberation of fluorophore, L.

The cytotoxicity of L toward the An-1 cells was investigated by the reduction activity of the methyl thiazolyl tetrazolium (MTT [3-(4,5-dimethyldihiazo-1-2-yl)-2,5-diphenyltetrazolium bromide]) assay. As shown in Fig. 9, the cellular viability was estimated to be approximately 89.5% after incubation with L (20 μM) for 24 h, which demonstrates that L has low cytotoxicity and biocompatibility to living cells. Due to the good

Fig. 10 Chemical structure of L.
spectroscopic properties and biocompatibility of the probe, L was applied for fluorescence imaging in living Ana-1 cells. As shown in Fig. 10, Ana-1 cells incubated with 5μM L for 30 min at 37°C gave no intracellular background fluorescence\(^{135}\) (Fig. 10a). Moreover, no intracellular fluorescence was detected for Ana-1 cells with 50μM FeCl\(_3\) in the growth medium for 3 h at 37°C. However, as shown in Fig. 10b, bright intracellular fluorescence was observed for Ana-1 cells with 50μM FeCl\(_3\) in the growth for 3 h at 37°C and then staining with L under the same loading conditions. After treating the cells with 25μM NaOAc for another 3 h at 37°C, the intense red fluorescence of the cells disappeared (Fig. 10c). It can be seen from the bright field transmission measurement after Fe\(^{3+}\) and L incubation (Fig. 10d) that the Ana-1 cells were viable throughout the imaging experiments. These facts implied that L was membrane-permeable and could be used for detecting Fe\(^{3+}\) in biological samples. 

![Fluorescence and phase contrast images of living Ana-1 cells](image)

Fig. 10 Fluorescence and phase contrast images of living Ana-1 cells: (a) fluorescence image of Ana-1 cell incubated with L (5μM) for 30 min at 37°C in the red channel; (b) fluorescence image of Ana-1 cell incubated with L (5μM) for 30 min, and then incubation with Fe\(^{3+}\) (50μM) for 3 h at 37°C in the red channel; (c) On the basis of panel b, 25μM of NaOAc for another 3 h at 37°C; (d) bright field image of living Ana-1 cells shown in panel b to confirm viability.

3. Experimental

3.1. Materials and instrumentation

All reactants and chemicals were obtained from Aladdin and used without further purification. Thin layer chromatography (TLC) was carried out on alumina backed plates coated with Merck gel GF254. Mass spectral data were obtained on a Solaril X70 FT-MS. \(^1\)H NMR and \(^1\)C NMR were recorded at room temperature on a Bruker Avance-500 NMR spectrometer. Chloroform (CDCl\(_3\)) was used as solvent and tetramethylsilane (TMS) as internal standard. Fluorescence spectra measurements were recorded with a Hitachi F-4500 spectrophotometer. A Techcomp UV-8500 spectrophotometer was used for Absorption measurements.

3.2. Synthesis and Characterization

The substituted benzaldehyde (3) was prepared according to the literature method \(^{36}\). The unique ligand L was prepared via the reaction of rhodamine hydrazide and 3 in methanol. (Scheme 1)

![Scheme 1. Synthetic route of L](image)

A solution of Rhodamine B (2.00 g, 4.18 mmol) in methanol (80 mL) was added Hydrazine hydrate (1.00 mL, 16.72 mmol), and the resulting mixture was stirred and refluxed for 4 h. The solution changed from dark purple to light orange. Then the mixture was cooled and solvent was removed under reduced pressure to give Rhodamine hydrazide. The crude Rhodamine hydrazide was dissolved in 80 mL absolute ethanol. Then intermediate 3 (12mmol) was added the mixture was refluxed for 6 h. The solvent was then removed and the crude product was purified by column chromatography on silica gel using dichloromethane/methanol=25:1 (v/v) as eluant to get compound L (1.12 g, 44.5%) as pink solid. \(^1\)H NMR (CDCl\(_3\), 500MHz, ppm): 8.42(s, 1H, CH=N), 7.98 (d, 1H, Ar-H), 7.51 (m, 2H, Ar-H), 7.37 (s, 1H, Ar-H), 7.30(d, 1H, Ar-H), 7.19 (d, 1H, Ar-H), 6.79 (d, 2H, Ar-H, J=8.5 Hz), 6.56 (d, 1H, Ar-H, J=5.5), 6.45(d, 2H, Ar-H, J=2.2 Hz), 6.27(d, 2H, Ar-H, J=2.2Hz), 6.0(m, 1H, =CH), 5.32 (s, 1H, OH), 5.14(d, 2H, =CH\(_2\)_J=6.5), 3.53(m,10H, NCH\(_2\)_CH\(_3\)_), 1.18 (t, 12H, NCH\(_2\)_CH\(_3\)_J=7.1Hz); \(^1\)C NMR (CDCl\(_3\), ppm): 12.61, 29.32, 35.05, 44.32, 65.92, 97.98, 106.15, 108.12, 115.78, 116.57, 123.30, 123.70, 125.28, 127.75, 128.22, 129.25, 129.70, 133.15, 136.12, 147.29, 148.95, 151.99, 153.05, 155.97, 164.89. FT-MS(ESI) m/z: calc for C\(_{38}\)H\(_{47}\)N\(_7\)O\(_6\) [M+H]\(^{+}\): 601.3127, found 601.3142;

4. Conclusion

In summary, a new fluorescent probe L was designed and synthesized. L exhibited extreme selectivity for Fe\(^{3+}\) over other metal ions in aqueous environment. Furthermore, the in situ generated nonfluorescent L-Fe\(^{3+}\) complex was employed for the specific recognition of AcO\(^-\) by fluorescence turn-off signaling through a displacement process. Confocal microscopy imaging implied that L can be potentially used as a promising candidate for Fe\(^{3+}\) and AcO\(^-\) imaging in Ana-1 cell.

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