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Fluorescence detection of Fe^{3+} ions in aqueous solution and living cells based on a high selectivity and sensitivity chemosensor

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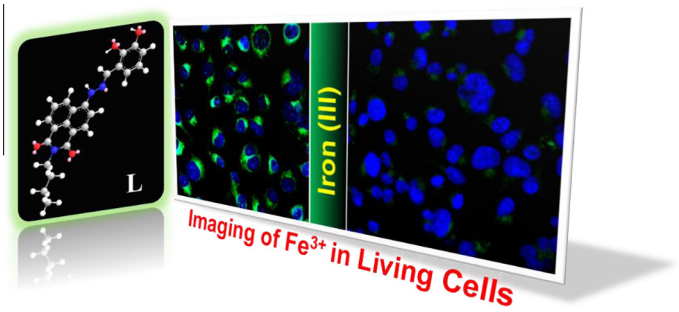
HIGHLIGHTS

- A highly sensitive and selective fluorescence chemosensor (**L**) was developed.
- **L** features high sensitive with the detection limit for Fe^{3+} ions was as low as 2 μM.
- **L** could be used for the imaging and quantification of Fe^{3+} ions in single intact cell.

GRAPHICAL ABSTRACT

A highly sensitive and selective fluorescence chemosensor, **L**, was developed for the detection of Fe^{3+} ions in aqueous solution and single intact cell.



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ABSTRACT

Although ferric ion (Fe^{3+}) performs critical roles in diverse biochemical processes in living systems, its physiological and pathophysiological functions have not been fully explored due to the lack of methods for quantification of Fe^{3+} ions in biological system. In this work, a highly sensitive and selective fluorescence chemosensor, **L**, was developed for the detection of Fe^{3+} ions in aqueous solution and in living cells. **L** was facile synthesized by one step reaction and well characterized by NMR, API-ES, FT-IR, and elementary analysis. The prepared chemosensor displayed excellent selectivity for Fe^{3+} ions detection over a wide range of tested metal ions. In the present of Fe^{3+} ions, the strong green fluorescence of **L** was substantially quenched. The 1:1 stoichiometry of the complexation was confirmed by a Job's plot. The association constant (K_a) of **L** with Fe^{3+} was evaluated using the Benesi–Hildebrand method and was found to be $1.36 \times 10^4\ M^{-1}$. The MTT assay determined that **L** exhibits low cytotoxicity toward living cells. Confocal imaging and flow cytometry studies showed that **L** is readily interiorized by MDA-MB-231 cells through an energy-dependent pathway and could be used to detect of Fe^{3+} ions in living cells.

Introduction

Ferric ion (Fe^{3+}), an essential trace element, plays significant roles in chemical and biological processes in living organisms [1,2]. In biological systems, Fe^{3+} ions mainly accumulates within

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liver, spleen and bone marrow cells, bound to ferritin [3] to provide oxygen-carrying capacity of heme and acts as a cofactor in many enzymatic reactions [4]. More specifically, it participates in many biological processes ranging from oxygen metabolism to DNA and RNA synthesis [5–7]. The deficiency of Fe^{3+} ions causes anemia, hemochromatosis, liver damage, diabetes, Parkinson's disease and cancer [8–11]. High levels of Fe^{3+} ions within the body have been associated with increasing incidence of certain cancers and dysfunction of certain organs, such as heart, pancreas and liver [12–16]. Therefore, development of the sensitive and selective detection approaches in biological systems is of great importance for deeper investigating of physiological and pathophysiological functions of Fe^{3+} in living organisms.

Recently, several of methods for the detection of Fe^{3+} ions have been reported, such as atomic absorption spectrometry, voltammetry, fluorometric and colorimetric method [13,17,18–20]. Among these methods, fluorometric assay with specific fluorescence chemosensor is currently attracting much attention as a method to reveal the molecular functions of the ions in living systems [21–23]. By combining with microscopy imaging, fluorescent chemosensor can be exploited as a powerful approach to investigate ions and biomolecules of interest with high temporal and spatial resolution in a noninvasive manner [24–31]. In addition, precise evaluation of ions in living cells could be realized with high sensitivity flow cytometry assay by utilizing designed florescence chemosensor [32,33]. Although much effort has been focused on the design of fluorescence chemosensor for the detection of Fe^{3+} ions in living systems, most of the prepared chemosensors exhibited poor selectivity toward other paramagnetic metal ions, such as Cu^{2+} and Ni^{2+} [34–50]. Therefore, we have investigated much time and effort to develop a new high sensitivity and selectivity fluorescent method for mapping Fe^{3+} ions in living cells.

In this work, we described a 1,8-naphthalimide-based fluorescence chemosensor (**L**) by a straightforward synthetic route [51]. The synthesized **L** was well characterized by NMR, API-ES, FT-IR and elementary analysis. The photophysical properties and recognition behaviors of **L** have been investigated in detail through fluorescence spectra, UV–Vis absorption spectra in HEPES–THF (7:3, v/v, pH = 7.4) solution. The characteristic fluorescent response of **L** benefits the intracellular imaging and quantitatively detection of Fe^{3+} ions in living MDA-MB-231 cells.

Experiment

Reagents and instruments

All reagents and solvents were of AR grade and used without further purification unless otherwise noted. 1,8-naphthalene anhydride, Hydrazine hydrate and 4-diethylaminosalicylaldehyde were purchased from Sinopharm Chemical Reagent Co., Ltd. (China); Fresh stock solution of metal ions (nitrate salts, except for chloride of Cu^{+} and Mn^{2+}, all 20 mM) in H_2O were prepared for further experiments. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Inova-400 spectrometer with chemical shifts reported as *ppm* (in CD_3Cl, TMS as internal standard). API-ES mass spectra were recorded on a HP1100LC/MSD spectrometer. Melt point was measured with a digital melting point apparatus WRS-2A (Shanghai Precision & Scientific Instrument Co., Ltd). FT-IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer equipped with a Nic-Plan Microscope. Elemental analyses (C, H and N) were performed on an Elementary Vario EL analyzer. Fluorescence spectra were determined with LS 55 luminescence spectrometer (Perkin Elmer, USA). The absorption spectra were measured with a Lambda 900 UV/VIS/NIR spectrophotometer (Perkin Elmer, USA). Fluorescent live cell images were acquired on an Olympus Fluoview FV 1000 IX81 inverted confocal laser-scanning microscope with an objective lens (×40). The excitation wavelength was 473 nm for imaging of **L**, and 405 nm for imaging of DAPI. The relative fluorescence intensities of images were analyzed by using an ImageJ software. Flow cytometric analysis was recorded on a BD FACSAria II flow cytometer with a laser at 488 nm. The data were analysed with Flowing software.

*Synthesis of compound **L***

To a solution of 4-hydrazine-1,8-naphthalimide [52] (0.14 g, 0.5 mmol) in methanol (20 mL), 1.2 equiv. of 2,3-dihydroxybenzaldehyde (0.083 g, 0.6 mmol) in 10 mL methanol was added with continuous stirring. Then, the mixture was refluxed for 3 h to form an orange turbid solution. After cooled to R.T., the precipitate was washed with methanol and dried under vacuum to get the orange solid **L** with the yield at 88.4%. Mp: 200.9–201.8 °C. ^{1}H NMR: (400 MHz, $CDCl_3$) 11.47 (s, 1H), 9.52 (s, 1H), 9.45 (s, 1H), 8.80 (s, 1H), 8.47(d, J = 6.0 Hz, 1H), 8.38 (d, J = 6.8 Hz, 1H), 7.78 (t, J = 6.2 Hz, 1H), 7.60 (d, J = 6.8 Hz, 1H), 7.27(d, J = 4.0 Hz, 1H), 6.83 (d, J = 5.6 Hz, 1H), 6.73 (t, J = 5.8 Hz, 1H), 4.01–4.04 (t, 2H), 1.58 (m, 2H), 1.31–1.34 (m, 2H), 0.90–0.93 (t, 3H). ^{13}C NMR: (100 MHz, $CDCl_3$) δ 164.22, 163.78, 155.42, 149.37, 137.16, 132.14, 131.31, 131.07, 129.00, 122.90, 122.73, 121.82, 118.34, 110.45, 105.70, 58.54, 49.36, 29.86, 18.45. IR (KBr, cm^{-1}): 3409, 2972, 2926, 1683, 1635, 1588, 1464, 1391, 1274, 1236, 1086, 1048. API-ES (negative mode, m/z) Calcd for $C_{23}H_{21}N_3O_4$: 403.15. Found: 402.20 (**L**−H$^+$). Anal. Calcd for: C 68.47; H, 5.25; N, 10.42; Found: C, 68.03; H, 5.29; N, 10.62.

Cation recognition studies by UV–Vis and fluorescence spectroscopy

Deionized water was used throughout all experiments. **L** (10 μM) was added with different metal ions (200 μM) in THF:HEPES (3:7, v/v, pH = 7.4) buffer solution. Excitation wavelength for **L** was 460 nm. The metal ions recognition behavior was evaluated from the change in spectrum of **L** upon addition of that metal salt. The light path length of cuvette was 1.0 cm.

Association constant calculation

Generally, for the formation of 1:1 complexation species formed by the receptor and the guest cation, the following Benesi–Hildebrand [53] equation was used to determining the association constants (K_a).

$$\frac{1}{F_0 - F} = \frac{1}{K_a(F_0 - F_{\min})[Fe^{3+}]} + \frac{1}{F_0 - F_{\min}}$$

where F_0 represent the fluorescence emission at 550 nm of **L** and F is the intensity in the presence of Fe^{3+} ions, $F_{\min}$ is the saturated fluorescence intensity of **L** in the presence of excess amount of Fe^{3+} ions; [Fe^{3+}] is the concentration of Fe^{3+} ions ion added, and K_a is the binding constant.

Quantum yield measurement

Fluorescence quantum yield was determined using optically matching solutions of fluorescein (Φ_f = 0.85 in 0.1 M NaOH) as standard at an excitation wavelength of 550 nm and the quantum yield is calculated using the equation [54]:

$$\Phi_{unk} = \Phi_{std}\frac{(F_{unk}/A_{unk})}{(F_{std}/A_{std})}\left(\frac{\eta_{unk}}{\eta_{std}}\right)^2$$

where Φ_{unk} and Φ_{std} are the radiative quantum yields of the sample and standard, F_{unk} and F_{std} are the integrated emission intensities of