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A rhodamine based fluorescent probe for Hg^{2+} and its application to cellular imaging

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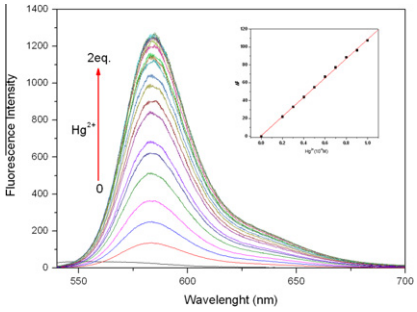
HIGHLIGHTS

- This work designed and synthesized a new fluorescent probe Rh-F based on rhodamine derivative.
- The probe Rh-F exhibits high selectivity and sensitivity in both absorbance and fluorescence detection of Hg^{2+}.
- Rh-F was successfully applied in Fluorescent imaging of Hg^{2+} in L-929 cells.

GRAPHICAL ABSTRACT



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ABSTRACT

A new rhodamine-based fluorescent probe (Rh-F) for detection of Hg^{2+} ions was synthesized, which could bind Hg^{2+} in aqueous ethanol (7:3, v/v) at pH 7.0 with detectable change in color and fluorescence. The response is based on a ring opening reaction and formation of a 1:1 complex, while ring-opening process of spirolactam enables large fluorescent enhancement and colorimetric change upon the addition of Hg^{2+}. The response is reversible and linear in the range between 200 nM and 1000 nM, with a detection limit of 4.2 nM. Selectivity and competition experiments with various other metal ion revealed that Rh-F possesses highly selective fluorescent response to Hg^{2+}. Furthermore, the probe was successfully applied to fluorescent imaging of Hg^{2+} in L-929 cells confirm that Rh-F can be used as a fluorescent probe for monitoring Hg^{2+} in living cells.

Introduction

The synthesis and development of fluorescent sensors for detection of target metal ions have attracted a great interest because of their importance in biology, catalysis and environment [1,2]. Among those metal ions, particular attention has been paid to mercury ions for their extreme toxicity. The extreme toxicity of mercury and their derivatives results from its high affinity for thiol groups in proteins and enzymes, leading to the dysfunction of cells and consequently causing health problems [3]. Moreover, mercury pollution remains a potential danger to human health and the environment because both elemental mercury and ionic mercury can be converted into methyl mercury by bacteria in the environment, which subsequently bioaccumulates through the food chain [4].

However, current approaches for monitoring heavy-metal ions (such as Hg^{2+} ions) in waste water are mainly based on costly, time-consuming methods like atomic absorption/emission spectroscopy or inductively coupled plasma mass spectrometry, which are not very convenient and handy for "in-field" detection [5–7]. These limitations have actually set off an enormous interest among chemists for the development of chemosensors for Hg^{2+}. Fluorescent probes have proven to be essential and powerful tools to monitor in vitro and in vivo biologically relevant species such as metal ions due to their advantages over the other analytical methods in

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terms of sensitivity, high selectivity, non-complicated sampling, non-destructive and real-time monitoring of the processes occurring at different time [8].

Rhodamine is an ideal platform for fluorogenic probe design due to its excellent spectroscopic properties of large molar extinction coefficients, high fluorescence quantum yields, and long absorption and emission wavelength elongated to the visible region [9]. By virtue of these fascinating properties, various rhodamine-based chromogenic and fluorogenic chemosensors for Hg^{2+} have been reported [10–26]. Generally, these sensors bearing soft chelators such as S, O or N atoms can bind with Hg^{2+}. Without Hg^{2+}, these sensors exist in a non-emissive spirocyclic form. Addition of Hg^{2+} leads to reversible coordination with the ligand groups, resulting in spirocycle opening along with switch-on fluorescence and significant color changes for "naked-eyes" detection. To date, many investigations are still in progress to search for cheaper, convenient and effective rhodamine derivatives.

In our previous paper, we described a rhodamine based fluorescence probe, which bearing two amide moieties, can monitor the intracellular Hg^{2+} level in living cells [26]. We learned that appropriate arrangements of O binding sites might be a choice to be parts of a selective receptor for the selective recognition of Hg^{2+}. Herein, we design and syntheses of the Rh-F through introduction the furan ring into the basic structure of Rh-d, in which the O atom of the furan ring may provide one more binding site. And the probe Rh-F behaves as a fluorescent Hg^{2+} probe with high sensitivity and selectivity in aqueous ethanol (7:3, v/v) at pH 7.0. Interestingly, this sensor showed reversible fluorescence response towards Hg^{2+} ions. Furthermore, Rh-F was successfully applied to fluorescent imaging of Hg^{2+} ions in L-929 cells.

Experiment

Materials and instruments

All the materials for synthesis were purchased from commercial suppliers and used without further purification. All reactions were monitored by TLC (thin-layer chromatography) with detection by UV. Deionized water was used throughout the experiment.

The absorption spectra were acquired on a Purkinje General TU-1901 UV–vis spectrometer. Fluorescence spectra measurements were performed on a Hitachi F-4500 fluorescence spectrophotometer at room temperature. ^{1}H-NMR and ^{13}C-NMR were measured on a Bruker Avance 300 MHz spectrometer with chemical shifts reported in ppm (in $CDCl_3$; TMS as internal standard). Electrospray ionization (ESI) mass spectra were conducted by ABI-057-TY4675 instrument. All pH were carried out on a PHS-3W pH meter. Fluorescence images of L-929 cells were carried out with an Olympus IX71 inverted fluorescence microscope.

General procedures of metal ion sensing

Two millimolar of each inorganic salt ($NaNO_3$, KNO_3, $Mg(NO_3)_2{\cdot}6H_2O$, $Mn(NO_3)_2{\cdot}4H_2O$, $Fe(NO_3)_3{\cdot}6H_2O$, $Ca(NO_3)_2$, $Zn(NO_3)_2{\cdot}6H_2O$, $Ba(NO_3)_2$, $Cu(NO_3)_2{\cdot}3H_2O$, $Pb(NO_3)_2$, $Cd(NO_3)_2{\cdot}2H_2O$, $AgNO_3$ and $HgNO_3{\cdot}0.5H_2O$) was dissolved in distilled water to afford 2×10^{-3} - mol L^{-1} aqueous solution. A 2.0×10^{-3} mol L^{-1} stock solution of Rh-F was prepared in absolute ethanol. All the measurements were made according to the following procedure. To 10 mL volumetric flask containing different amounts of metal ions, proper amounts of the solution of Rh-F was added directly with micropipette, then diluted with buffered (HEPES 20 mM, pH = 7.0) aqueous-ethanol (7/3, v/v) to 10 mL, then the absorption and fluorescence sensing of metal ions were run. In selectivity experiments, the test samples were prepared by placing the appropriate amounts of metal ion stock solution into 10 mL solution of Rh-F (10 μM). Fluorescence and UV–vis spectra were measured after addition of Hg^{2+} for 15 min at room temperature to equilibrium. Fluorescence measurements were carried out with excitation and mission slit width of 10 and 5 nm and excitation wavelength was 520 nm.

Synthesis of probe Rh-F

The synthesis of probe Rh-F is depicted in Scheme S1. Rhodamine ethylenediamine (3) was facilely synthesized in high yield according to the procedures reported in the literature [27]. Furyl acryloyl chloride (2) was synthesized according to the reported procedures [28].

Briefly, rhodamine ethylenediamine (3) (2.8 g, 5.8 mmol) and triethylamine (1.7 mL) were dissolved in 40 mL dry dichloromethane and stir in ice bath. Furyl acryloyl chloride (2) (0.94 g, 6 mmol) in 10 mL of dichloromethane was added dropwise to the above solution. The ice bath was removed and the solution was stirred 2 h at room temperature. The result mixture was washed with water (40 mL × 2) and the organic phase was dried over anhydrous sodium sulfate. Then the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (silica gel, petroleum ether: ethyl acetate 1:1) affording 2.63 g of Rh-F in 75% yield.

IR (KBr, ν/cm^{-1}): 3398.60, 2970.14, 2871.80, 1671.84, 1614.83, 1468.37, 1221.00, 1117.58, 969.60. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.92 (dd, J = 5.5, 3.0 Hz, 1H), 7.60–7.39 (m, 3H), 7.30 (d, J = 15.5 Hz, 1H), 7.09–7.08 (m, 2H), 6.25–6.51 (m, 9H), 3.37–3.19 (m, 12H), 1.17 (t, J = 6.9 Hz, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.01, 165.71, 153.84, 153.31, 151.64, 148.96, 143.63, 132.81, 130.50, 128.48, 128.20, 127.10, 123.93, 122.86, 119.25, 113.05, 111.98, 108.36, 104.81, 97.88, 65.74, 44.41, 12.86. ESI-MS ($M + H^+$): m/z = 604.30 ($C_{37}H_{40}N_4O_4$).

Binding constant

The data obtained from fluorescence titration profile were fitted to be a 1:1 binding model according to the following equation.

$$Y = Y_o + \frac{Y_{\lim - Y_0}}{2}\left\{1 + \frac{c_M}{c_L} + \frac{1}{Kc_L} - \left[\left(1 + \frac{c_M}{c_L} + \frac{1}{Kc_L}\right)^2 - 4\frac{c_M}{c_L}\right]^{\frac{1}{2}}\right\} \quad (1)$$

The association constant (K) is an important parameter, indicating the inclusion capacity of the host–guest complex. The association constant (K) can thus be obtained by a nonlinear least's squares analysis of ΔF versus [Hg^{2+}], fitting to the experimental data obtained from the fluorescence titrations. Y was the recorded fluorescent intensity, Y_0 was the start value without the addition of target molecule, $Y_{\lim}$ was the limiting value (left as a floating parameter), C_M was the target molecule concentration, and C_L was the sensor concentration.

Cell culture and fluorescence imaging

The L-929 cells were cultured in DEME (Invitrogen) supplemented with 10% FBS (Invitrogen). One day before imaging, the cells were seeded in 6-well flat-bottomed plates. The next day, the L-929 cells were incubated with 10 μM Rh-F for 0.5 h at 37 °C in humidified environment of 5% CO_2 and then washed with phosphate-buffered saline (PBS) three times to remove the remaining sensor. Before incubating with 10 μM $Hg(NO_3)_2$ for another 0.5 h, cells were rinsed with PBS three times again, then the fluorescence imaging of intracellular Hg^{2+} was observed under