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Rhodamine-based fluorescent sensor for mercury in buffer solution and living cells

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ABSTRACT

A novel fluorescent sensor based on thiooxorhodamine B has been prepared to detect Hg^{2+} in aqueous buffer solution. It demonstrates high selectivity for sensing Hg^{2+} with about 383-fold enhancement in fluorescence emission intensity and micromolar sensitivity ($K_d = 7.5 \times 10^{-6}$ mol L^{-1}) in comparison with alkali and alkaline earth metal ions (K^+, Na^+, Mg^{2+}, Ca^{2+}) and other transition metal ions (Mn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ag^+, Pb^{2+}, Cr^{3+}, Fe^{3+}). Meanwhile the distinct color changes and rapid switch-on fluorescence also provide 'naked eyes' detection for Hg^{2+} over a broad pH range. Moreover, such sensor is cell-permeable and can visualize the changes of intracellular mercury ions in living cells using fluorescence microscopy.

1. Introduction

Toxic heavy metals such as mercury, cadmium and lead can cause lethal threat to the environment and human beings. In particular, mercury has been drawing extensive attention due to its bioaccumulation in organism in the form of methyl mercury and transportation in the soluble form of mercury ion [1]. These accumulations consequently cause serious diseases such as prenatal brain damage, serious cognitive, motion disorders and minamata disease [2]. Therefore, much effort has been devoted to develop new sensitive and selective detecting method for toxic and hazard mercury.

Owing to the facile measurement, rapid detection and less-cost, fluorogenic and chromogenic sensors are excellent candidates to probe mercury in the environmental and physiological samples [3]. Among these sensors, rhodamine and its derivatives have been widely employed to design fluorescent sensors due to their good photostability, high extinction coefficient and high fluorescence quantum yield [4].

Accordingly, some rhodamine-based Hg^{2+}-selective sensors have been envisaged by utilizing the Hg^{2+}-induced desulfurization effect [5]. The sensing process is irreversible, in which the thiocarbonyl groups are desulfurized to yield oxocarbonyl or cyclization products simultaneously resulting in the spiro-ring opening rhodamine derivatives with switch-on fluorescence and significant color changes for "naked-eyes" detection. On the other hand, Hg^{2+}-coordination induced ring-opening process is also applied to develop Hg^{2+} sensors [6]. Generally, these sensors bearing 'soft' chelators such as S or N atoms can selectively bind with 'soft' Hg^{2+}. Consequently, such coordination leads to the opened-ring form and significant signal output. However, more improvements for these rhodamine-based sensors are still in demand to be compatible with biological and environmental applications due to the lack of insufficient selectivity, sensitivity, water solubility and pH independence.

It has been reported that the thiospirolacton rhodamine derivative is an ideal chromophore to construct reversible sensors and can undergo direct spiro-ring opening process due to the coordination between Hg^{2+} and S atom in acidic conditions [7]. Further combination of such chromophore and 'soft' chelator units provides high affinity for Hg^{2+} under physiological conditions [8]. These findings encouraged us to carefully select an appropriate binding unit so as to generate suitable coordination sites towards Hg^{2+} and spatial effect within a molecule by simple structural modification. This would afford a fluorescent sensor with high

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affinity, sensitivity, and fast detection for Hg^{2+}. To this end, we designed the sensor ThioRh-1. This sensor turns out to be an efficient Hg^{2+}-selective fluorescent sensor in a HEPES-buffer solution with high sensitivity and selectivity. Moreover, we also report the application for detection of Hg^{2+} in living cells.

2. Experiment procedures

2.1. General

All titrations were carried out in HEPES buffer (1×10^{-2} mol L^{-1} HEPES, 1×10^{-1} M $NaClO_4$, pH = 7.4, 50% ethanol, v/v). UV-vis and fluorescence spectra were recorded on HITACHI 3010 UV-vis spectrometer and HITACHI F-4600 spectrometer, respectively. All ^1H and ^{13}C NMR spectra were measured on Bruker AVANCE-400 400 MHz spectrometer. HRMS-ESI was measured on Bruker Apex IV Fourier transform mass spectrometer.

2.2. Synthesis

Thiooxorhodamine B hydrazide (1.0 mmol, 0.47 g) was dissolved in dry ethanol (10 ml). 2,3-butanedione (2.0 mmol, 0.17 g) and 3 drop acetic acid were added to the solution. The reaction mixture was refluxed overnight under N_2 atmosphere. The yellow precipitates were filtered and washed with cold ethanol. The crude product recrystallized from hot EtOH to afford a yellowish solid ThioRh-1 (0.46 g, yield: 85%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 7.5 Hz, 1H), 7.47 (d, J = 7.1 Hz, 2H), 7.16 (d, J = 6.6 Hz, 1H), 6.76 (d, J = 8.7 Hz, 2H), 6.43–6.22 (m, 4H), 3.36 (d, J = 6.5 Hz, 8H), 2.44 (s, 3H), 2.24 (s, 3H), 1.17 (t, J = 6.8 Hz, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.93, 172.50, 161.56, 155.49, 152.17, 148.54, 135.47, 132.89, 130.14, 128.20, 127.29, 122.89, 110.06, 108.43, 97.70, 77.59, 77.27, 76.96, 64.32, 44.55, 25.15, 12.82, 11.75. TOF-MS: m/z 541.4 $[M + H]^+$. HRMS (ESI) Calcd for $C_{32}H_{37}N_4O_2S$, 541.26317; Found, 541.26262.

2.3. General procedure for Job's plot

A series of solutions containing ThioRh-1 and $HgCl_2$ were prepared such that the sum of the total metal and ThioRh-1 concentration remained constant at 1×10^{-5} mol L^{-1}. The molar fraction x of ThioRh-1 was varied between 0.1 and 1.0. The fluorescence intensity at 591 nm was plotted against the molar fraction of the sample solution.

2.4. Determination of dissociation constant

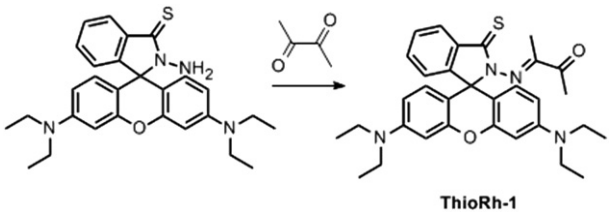
Fluorescence intensity at 591 nm of 5×10^{-6} mol L^{-1} ThioRh-1 as a function of Hg^{2+} concentrations was measured in HEPES buffer solution. The solutions were allowed to equilibrate at 25 ± 0.5 °C for 3 min after each addition. The fluorescence intensity (F_{591nm}) was plotted and fitted to the following equation (1) with 1:1 binding mode.

$$F = \frac{\left[M^{2+}\right]F_{max} + K_d F_{min}}{K_d + \left[M^{2+}\right]} \quad (1)$$

where F is fluorescence intensity, K_d is dissociation constant, F_{min} is fluorescence intensity of the free ligand, F_{max} is fluorescence intensity of the mercury-loaded sensor, and $[M^{2+}]$ is mercury concentration.

2.5. Cell incubation

A549 cell was used for fluorescence imaging. The cells were incubated in Dulbecco's modified Eagle's medium (DMEM, Hyclone) supplemented with 10% fetal calfserum (Hyclone), penicillin/streptomycin (100 μg/mL, Hyclone) at 37 °C in a 5:95 CO_2-air incubator. The cells were cultured for 3 days, then loaded on a 35 mm diameter glass-bottomed coverslips. The cells were incubated with 1×10^{-5} mol L^{-1} ThioRh-1 for 30 min in incubator, washed with PBS three times and bathed in PBS (2×10^{-3} L) before imaging.



Scheme 1. Synthesis of sensor ThioRh-1.

2.6. Confocal fluorescence microscopy

Olympus FV-1000 laser scanning microscopy system equipped with a 515 nm laser head was applied to confocal image A549 cell stained with ThioRh-1. Emission was collected at 560 ~ 610 nm. All images were gathered at the same confocal microscope settings and processed with Olympus FV10-ASW Ver. 2.1 software (Olympus, Japan). ThioRh-1 was added to A549 cells in the coverslips that contained 2×10^{-3} L culture medium (serum-free), and was incubated at 37 °C for 30 min. After removing the culture medium and washing with PBS three times, the fluorescence images of cells in PBS were taken. Bright-field images confirmed the viability of the cells during the experiment. Then 3×10^{-5} mol L^{-1} of Hg^{2+} was added, which the cells were imaged every 5 min within one hour.

3. Results and discussions

As shown in Scheme 1, one-pot condensation of 2,3-butanedione with thiooxorhodamine B hydrazide afforded ThioRh-1. The detailed synthesis and characterization were listed in the experimental section and supporting information.

The UV/Vis titration absorption spectra of ThioRh-1 (1.25×10^{-5} mol L^{-1}) in HEPES buffer (1×10^{-2} mol L^{-1} HEPES, 1×10^{-1} M $NaClO_4$, pH = 7.4, 50% ethanol, v/v) is depicted in Fig. 1.

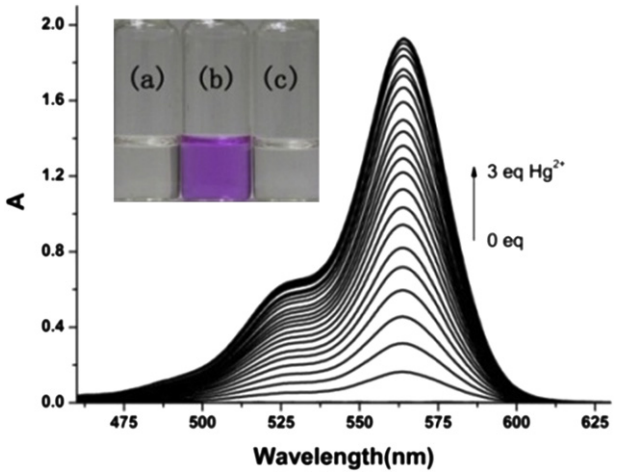


Fig. 1. UV/Vis absorption spectra of ThioRh-1 (1.25×10^{-5} mol L^{-1}) with addition of mercury ions (0–3 equiv). Inset: color changes of ThioRh-1 in the visible range. (a): ThioRh-1 in buffer; (b): (a) $+Hg^{2+}$; (c): (b) $+Na_2S$.

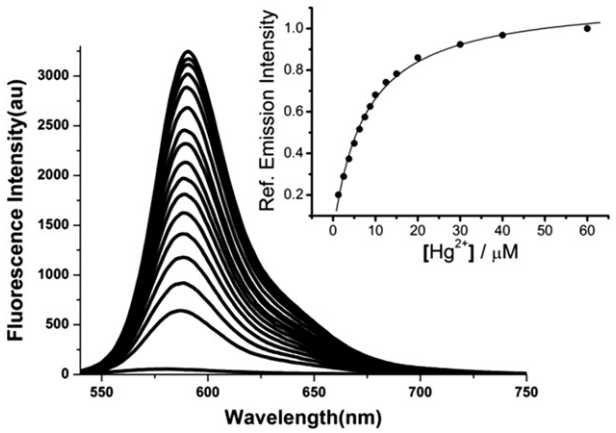


Fig. 2. Fluorescence emission spectra ($\lambda_{ex} = 510$ nm) of ThioRh-1 (5×10^{-6} mol L^{-1}) upon addition of mercury (0–3 equiv) in aqueous buffer. Inset: fluorescence intensity at 591 nm plots against [Hg^{2+}] according to fluorescence titration spectra.

The sensor ThioRh-1 exhibits very weak absorption in the visible range in the absence of Hg^{2+}. It can be explained that ThioRh-1 exists in the form of spirocyclic structure in the solution. Addition of Hg^{2+} to ThioRh-1 leads to an intense change in the visible range. Upon addition of 3 equiv Hg^{2+}, a distinct absorption band centered at 563 nm was observed (Fig. 1). Meanwhile, the colorless solution of ThioRh-1 rapidly turned into red due to the ring-opening process by interacting with Hg^{2+}. Subsequent addition of Na_2S resulted in colorless solution, implying a reversible coordination process between ThioRh-1 and Hg^{2+} rather than a desulfurization one. The desulfurization mechanism is commonly utilized to design fluorescent sensors for mercury ions but not in the present case [5]. Both the color and spectra changes indicate that ThioRh-1 can probe mercury reversibly and this detection process can be easily observed by naked eyes (Fig. 1, Inset).

Next we evaluated fluorescence emission property of ThioRh-1 (5×10^{-6} mol L^{-1}) in HEPES buffer. As shown in Fig. 2, when excited at 510 nm, the addition of 3 equiv Hg^{2+} triggered remarkable fluorescence emission enhancement ($F/F_0 = 383$, measured as fluorescence emission intensity at 591 nm). The coordination process was complete after the addition of Hg^{2+} within 3 min, implying that ThioRh-1 could be used for real-time detection of Hg^{2+} [8a]. Subsequent treatment with 3 equiv Na_2S, the fluorescence intensity could also be reversed to the initial value. Further

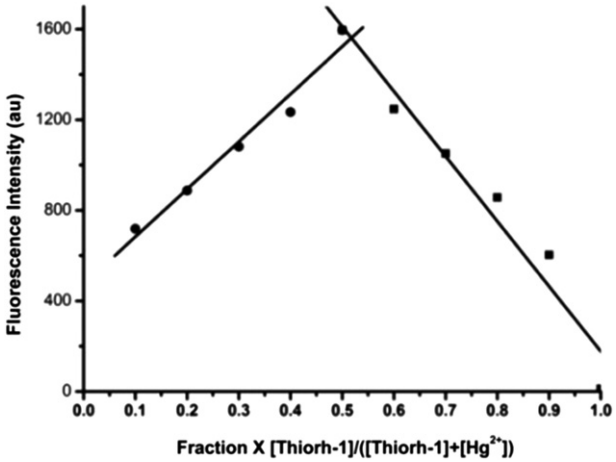
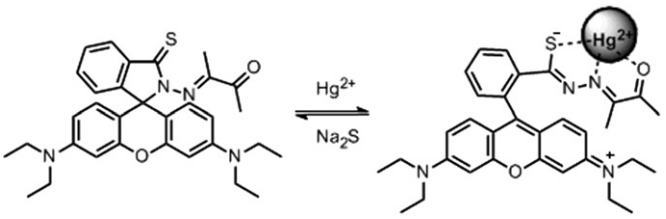


Fig. 3. Job's plot, the total concentration of ThioRh-1 and Hg^{2+} is 1×10^{-5} mol L^{-1}.



Scheme 2. Possible binding mode of ThioRh-1 with Hg^{2+}.

Job's plot indicates that ThioRh-1 and mercury ion form 1:1 adduct (Fig. 3). This was further confirmed by the appearance of a peak at m/z 777.1928 (calcd for 777.1954) assignable to [ThioRh-1 + Hg^{2+} + Cl^-]$^+$ in the HRMS-ESI spectrum. Compared to the thiospirolacton rhodamine derivative-mercury 2:1 adduct, we infer that this 1:1 binding mode may origin from the steric hindrance effect of the two methyl groups. Presumably, this would confine the configuration of the complex. The possible binding mode between ThioRh-1 and Hg^{2+} was proposed as shown in Scheme 2. According to the fluorescence titration curve (Fig. 2, Inset), the binding constant was calculated to be $K_d = 7.5 \pm 0.6$ μM via nonlinear least-squares fitting ($R = 0.995$). Therefore, ThioRh-1 exhibits the significant Hg^{2+}-induced emission enhancement and high affinity

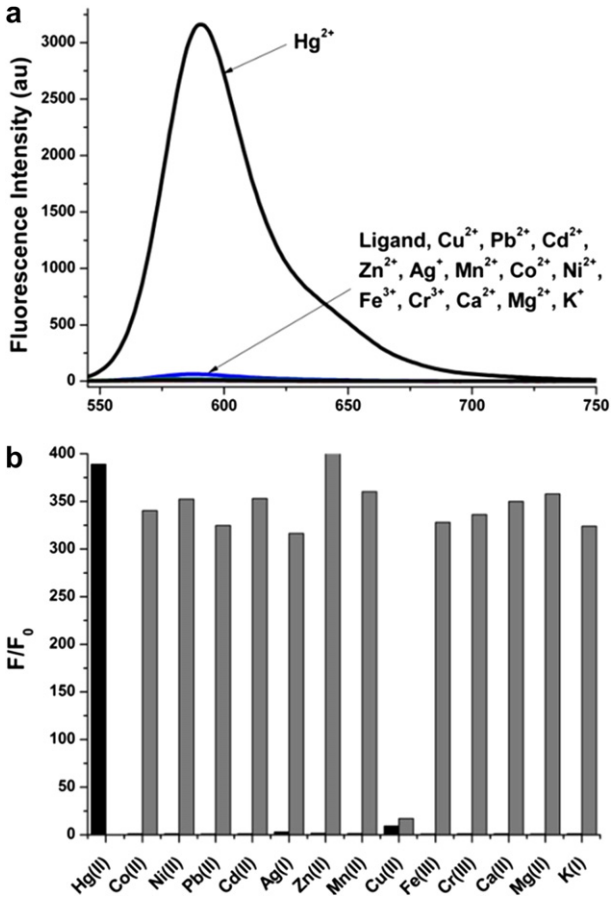


Fig. 4. (a) The fluorescence spectra of ThioRh-1 (5×10^{-6} mol L^{-1}) upon addition of 3 equiv various metal ions in the buffer. (b) Metal ion selectivity of ThioRh-1. The black bars represent the emission intensity of Thiorh-1 in the presence of 3 equiv Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+} and 100 equiv Mg^{2+}, Ca^{2+} and K^+; The gray bars represent the emission intensity of ThioRh-1 in the presence of the indicated metal ions, followed by 3 equiv Hg^{2+}.

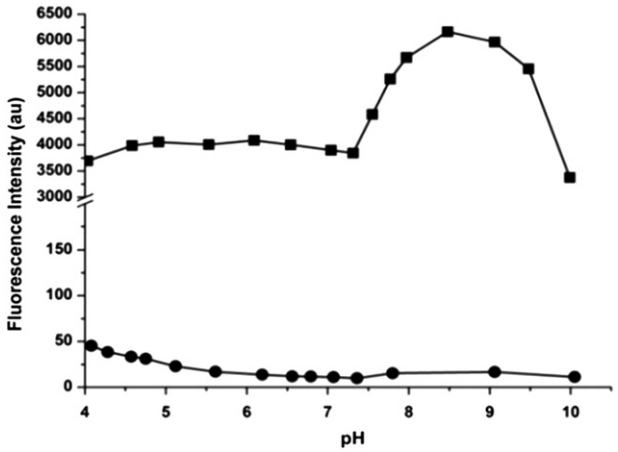


Fig. 5. Fluorescence intensity ($\lambda_{ex} = 510$ nm, $\lambda_{em} = 591$ nm) of 5×10^{-5} mol L^{-1} ThioRh-1 and its Hg^{2+}complex at various pH values in buffer solution.

for Hg^{2+}, and thus provides a good opportunity to detect Hg^{2+} with high sensitivity.

In addition, the selectivity profiles of ThioRh-1 for mercury were investigated by fluorimetric experiments. In the presence of other metal ions, such as alkali and alkaline earth metal ions (K^+, Na^+, Mg^{2+}, Ca^{2+}) and other transition metal ions (Mn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ag^+, Pb^{2+}, Cr^{3+}, Fe^{3+}), there was no evident fluorescence intensity enhancement (Fig. 4a). Apparently, due to Hg^{2+}-induced significant fluorescence enhancement, Hg^{2+} could be distinguished from other metal ions. To further gauge selectivity over other metal ions, M/Hg^{2+} coexisted systems were also examined. As shown in Fig. 4b, other metal ions except Cu^{2+} have posed a negligible effect on the fluorescence response of ThioRh-1 for Hg^{2+}. Cu^{2+} may also get involved in the coordination with ThioRh-1 and may consequently leads to a opened-ring product. However, only slight fluorescence enhancement was observed due to the quenching effect of the paramagnetic Cu^{2+} [9]. Obviously, ThioRh-1 displays distinct switch-on fluorescence emission for Hg^{2+} in contrast with that for Cu^{2+}.

Furthermore, we evaluated the influence of pH factor on ThioRh-1 and its mercury complex, the pH dependent fluorescence emission spectra changes of ThioRh-1 and its mercury complex are shown in Fig. 5. ThioRh-1 exhibits very weak fluorescence ranging from pH = 4.0 ~ 10.0. However, it rapidly responds to Hg^{2+} with distinct fluorescence enhancements over the same pH range. This evidence implies that ThioRh-1 can be used to detect mercury in either acid or base solutions with high sensitivity.

The properties of ThioRh-1 render it suitable for biological applications. We next evaluated the response of ThioRh-1 to Hg^{2+} in living cells. A549 cells were incubated with 1×10^{-5} mol L^{-1} ThioRh-1 in Dulbecco's modified Eagle's medium supplemented with 10% fetal calfserum, penicillin/streptomycin (100 μg/mL) at 37 °C in a 5:95 CO_2-air incubator. Then the cells were washed with PBS to remove the remaining ThioRh-1. As shown in Fig. 6(b), no evident fluorescence

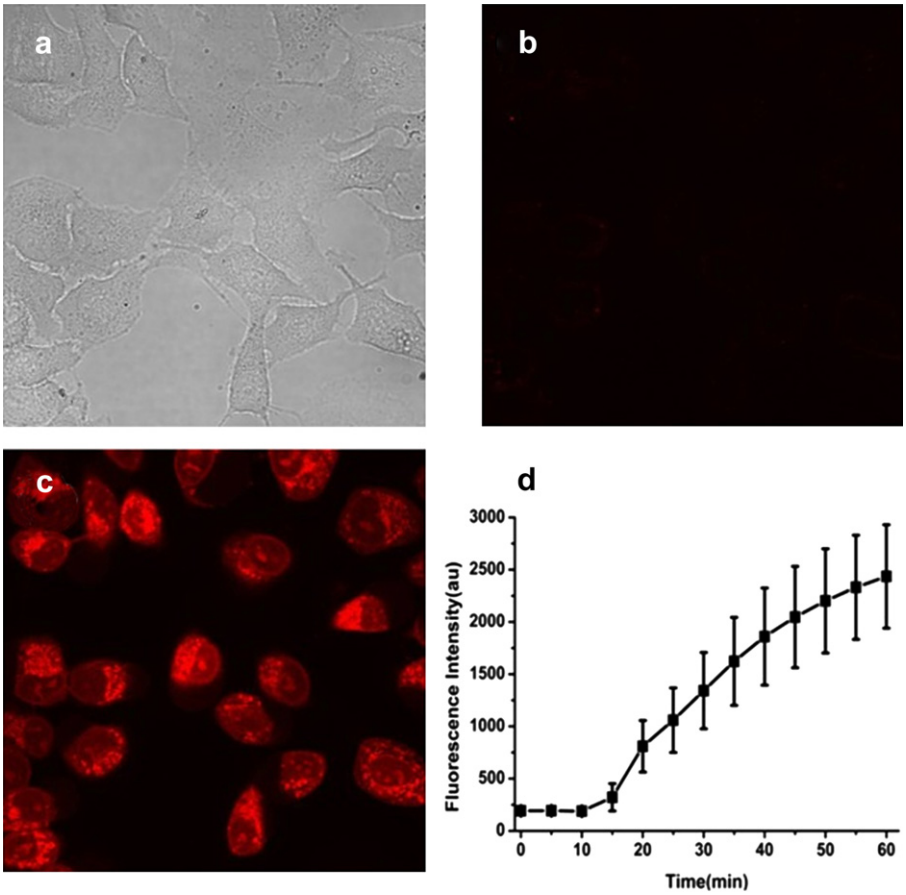


Fig. 6. Confocal fluorescence images of intracellular Hg^{2+} in A549 cells with ThioRh-1. (a) Bright-field transmission image. (b) Confocal fluorescence images stained with 1×10^{-5} mol L^{-1} ThioRh-1 at 560–610 nm. (c) Subsequently exposed to 3×10^{-5} mol L^{-1} Hg^{2+} for 40 min. (d) Averaged fluorescence intensity collected from 20 cells as a function of time.

was observed in the optical window at 560 ~ 610 nm. After treating with 3×10^{-5} mol L^{-1} Hg^{2+} for 40 min as displayed in Fig. 6(c), the fluorescence intensity increased dramatically. To measure the real-time response to Hg^{2+}, we have imaged the cells and recorded the fluorescence intensity every 5 min within one hour (Fig. 6(d) and S1). It displayed sensitive response to Hg^{2+} in living A549 cells. Thus ThioRh-1 is cell-permeable and can visualize the changes of intracellular Hg^{2+} in living cells.

4. Summary

In summary, a rhodamine-based Hg^{2+}-selective sensor, ThioRh-1, has been prepared on the basis of mercury's thiophilicity. This rhodamine-derived sensor can detect Hg^{2+} by both distinct color changes and switch-on fluorescence. Moreover, this sensor shows a broad working pH range for response to Hg^{2+}. Confocal microscopy experiments indicated that cell-permeable ThioRh-1 can visualize the changes of intracellular Hg^{2+} in living cells. This work also implies that simple structural modification of thiospirolacton rhodamine can easily contribute to new Hg^{2+}-selective fluorescent sensors.

Acknowledgements

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Appendix. Supplementary data

Characterization of ThioRh-1, and additional spectroscopic data associated with this article can be found in the online version, at doi:10.1016/j.dyepig.2011.04.007.

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