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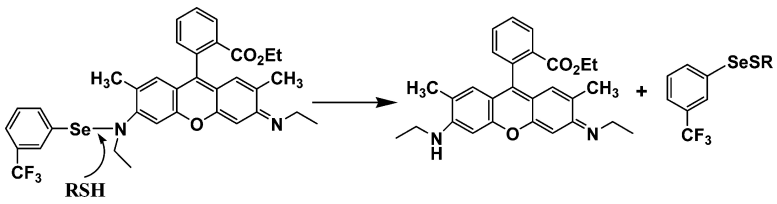

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A Rhodamine-Based Fluorescent Probe Containing a Se−N Bond for Detecting Thiols and Its Application in Living Cells

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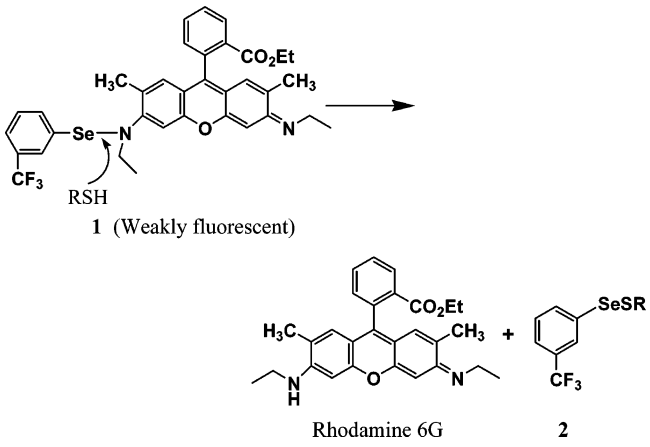
Thiols, which are components of many proteins and simple molecules, such as glutathione (GSH) and cysteine (Cys), play an important role in the cellular antioxidant defense system.[1] GSH is the most abundant intracellular nonprotein thiol (1−10 mM).[2] It has a pivotal role in maintaining the reducing environment in cells and acts as the redox regulator because thiols exist in redox equilibrium between sulfhydryl and disulfide forms.[3−5] Intracellular thiol levels change dramatically in the response to oxidative stress.[1] Thus, the quantitative detection of intracellular thiols is of great importance for investigating cell functions.

Among the reported methods for detecting thiols, the use of fluorescent probes had its apparent advantages over other methods in sensitivity and, most importantly, in imaging thiols in vivo, even in single living cells. Although high-performance liquid chromatography (HPLC) combined with Ellman's reagent (DTNB) is widely used for detecting thiols in a chemical system,[6] the method is inconvenient to operate and unsuitable for intracellular thiol detection. Bromobimanes as thiol-labeling fluorescent probes are also nonoptimal for intracellular applications owing to bromobimane-induced cell damage.[7] The fluorescent probe recently reported by Maeda et al.[8] was used in thiol quantification enzyme assays, but the probe suffers from the drawbacks of slight hydrolysis and the difficulty of application in vivo. As far as we know, only two imaging studies on thiols or GSH have been reported. One presented fluorescent dithiol probes for detecting thiols based on a redox mechanism and obtained fluorescence images of thiols in zebrafish embryos.[4] The other used combinatorial rosamine library fluorescent probes to monitor changes of GSH concentration in live 3T3 cells.[9] Although the two fluorescent probes have made great progress in imaging intracellular thiols or GSH, more effective probes with good sensitivity, selectivity, and practicability in living cells still need to be developed.

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2*H*)-one), the well-known mimetic of glutathione peroxidase (GPx) which is a key selenoenzyme in the biological antioxidant defense system,[10,11] exhibits its therapeutic properties for many diseases and is of vital importance in biology and medicine due to its low toxicity.[12] The catalytic GPx activity of this selenium-containing drug was explained,[13] which inspired us to design a fluorescent probe with a selenium−nitrogen (Se−N) bond to probe thiols. In our work, we synthesized a novel rhodamine-based fluorescent probe (Scheme 1, **1**) containing a Se−N bond for thiols. Our strategy is based on the strong nucleophilicity of the sulfhydryl to cleave the Se−N bond of probe **1** which is weakly fluorescent in aqueous solution, resulting in the formation of the corresponding selenenyl sulfide (Scheme 1, **2**) and the strongly fluorescent dye rhodamine 6G.

GSH was selected as the representative thiol in the spectral experiments. The spectral properties of probe **1** were tested by its reaction with GSH in simulated physiological condition (pH 7.4, 20 mM phosphate buffered saline (PBS)). As shown in the fluorescence spectra, λ_{max} of excitation and emission of **1** was seen at 525 and 550 nm, respectively (see Supporting Information, SI). When **1** (0.50 μM) reacted with different concentrations of GSH, there was a good linearity between relative fluorescence intensity (RFI) and concentrations of GSH in the range of 3.0×10^{-9} to 1.2×10^{-7} M (Figure 1), and the regression equation was $(F - F_0) = 281.4 + 770.1 \times$ [GSH] ($\times 10^{-7}$ M) with a linear coefficient of 0.9946, and the limit of detection of the proposed method was 1.4 nM, which indicated that **1** could detect GSH qualitatively and quantitatively. We next studied the effect of interference of a variety of biorelevant analytes on monitoring GSH. The results showed

Scheme 1. Reaction Mechanism of **1** and RSH



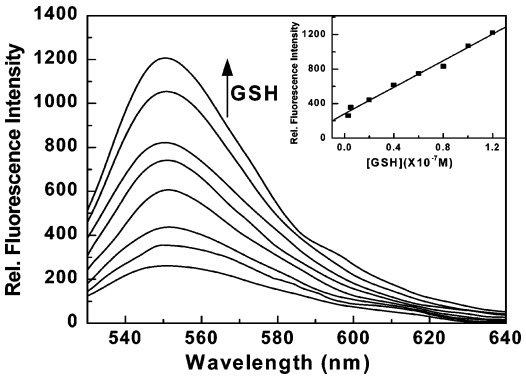


Figure 1. Fluorescence responses of probe **1** (0.50 μM) toward different concentrations of GSH (final concentration: 0.0030, 0.0050, 0.020, 0.040, 0.06, 0.080, 0.10, 0.12 μM) after incubation at 25 °C for 30 min in PBS (pH 7.4, 20 mM). Spectra were obtained at 550 nm (λ_{ex} = 525 nm) and a scan range of 530−640 nm.

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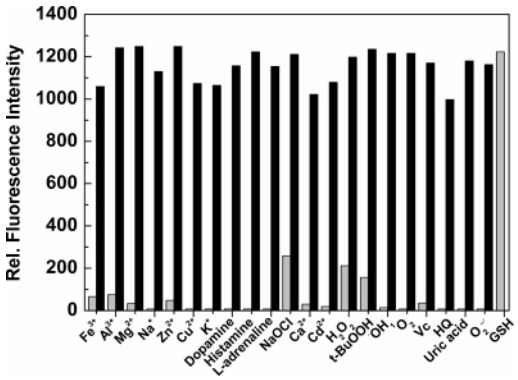

Figure 2. Fluorescence responses of **1** (0.50 μM) to diverse bioanalytes in PBS (pH 7.4, 20 mM). Light gray bars represent the addition of analytes: Fe^{3+} (0.36 mM); Al^{3+}, Mg^{2+}, Na^+, Zn^{2+} (0.18 mM); Cu^{2+}, K^+, dopamine, histamine, L-adrenaline, NaOCl (0.12 mM); Ca^{2+}, Cd^{2+}, H_2O_2 (60 μM); *t*-BuOOH, ·OH, $^1O_2$, ascorbic acid (Vc), hydroquinone (HQ) (36 μM); uric acid (24 μM); O_2^- (6.0 μM); GSH (0.12 μM). Black bars represent the subsequent addition of 0.12 μM GSH to the mixture. All data were obtained after incubation at 25 °C for 30 min ($\lambda_{ex}/\lambda_{em}$ = 525/550 nm).

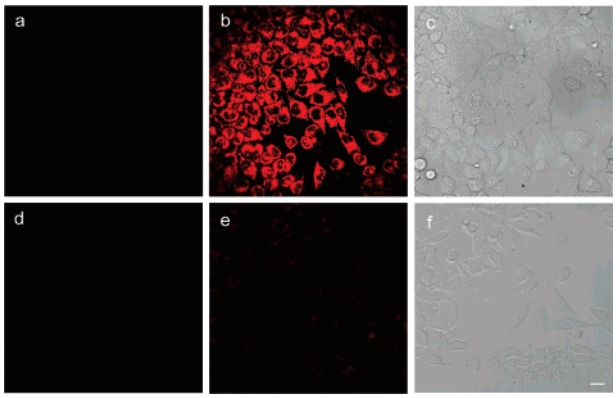

Figure 3. Confocal fluorescence images of living HL-7702 and HepG2 cells: HL-7702 cells incubated with probe **1** (0.50 μM) for (a) 15 min and (b) 30 min; HepG2 cells incubated with **1** (0.50 μM) for (d) 15 min, (e) 30 min; (c) and (f) represent the bright-field images of (b) and (e), respectively. Incubation was performed at 37 °C under a humidified atmosphere containing 5% CO_2. Scale bar = 20 μm.

that probe **1** possesses high selectivity toward GSH when present with other analytes (Figure 2).

Then, the reactivity of probe **1** to non-protein thiols and protein thiols was tested by measuring the increase in fluorescence intensity at 550 nm (SI). Probe **1** (0.5 μM) showed greater fluorescence response to GSH than other non-protein thiols such as 2-mercaptoethanol, *N*-acetylcysteine, Cys, and dithiothreitol (each 5 mM), while the responses of **1** toward each kind of detected protein thiols, including thioredoxin, glutathione reductase, and metallothionein (the detected concentrations were determined according to separate intracellular contents, SI), could reach nearly 2−3-fold higher than that of GSH. Taken together, probe **1** is more sensitive to protein thiols than non-protein thiols.

Normal hepatocytes conserve high levels of GSH and protein thiols, while liver injury caused by oxidative stress can induce depletion of GSH and severe decline in concentration of protein thiols.[14,15] To test the capability of **1** to image thiols in living cells, we further applied **1** to both the human normal liver cell line (HL-7702) and human hepatoma cell line (HepG2). After being incubated with **1** (0.50 μM) for 15 min, both kinds of cells nearly had no fluorescence (Figure 3a,d). However, when incubated with **1** (0.50 μM) for 30 min, HL-7702 cells showed a clear increase in the intracellular fluorescence intensity (Figure 3b), while HepG2 cells only showed faint fluorescence (Figure 3e). The bright-field images (Figure 3c,f) confirmed that the cells are viable throughout the imaging experiments. The results clearly demonstrated that **1** was able to sense the differences in thiol concentrations between HL-7702 and HepG2 cells, which are in accordance with the different fluorescence responses observed in cell extracts (SI). Further studies were performed by pretreatment of cells with the thiol-blocking reagent *N*-ethylmaleimide;[16] distinct decrease of fluorescence intensity in HL-7702 cells was observed, while no obvious change occurred in HepG2 cells (SI). The result confirmed that the probe is membrane-permeable, and the fluorescence changes in the HL-7702 cells were really due to the changes in the intracellular thiol levels.

In summary, we have developed a novel rhodamine-based fluorescent probe containing a Se−N bond for thiols based on the nucleophilic substitution of sulfhydryl. The new probe was successfully applied to the imaging of thiols in both HL-7702 cells and HepG2 cells with high sensitivity and selectivity.

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Supporting Information Available: Synthetic and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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