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A new fluorescence turn-on probe for biothiols based on photoinduced electron transfer and its application in living cells

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ABSTRACT

A new biothiol-selective fluorescent probe **1** based on photoinduced electron transfer (PET) mechanism was designed and synthesized. The UV–Vis absorption and fluorescent emission properties of probe **1** towards various analytes were studied in detail. The probe exhibited a large stokes shift (~200 nm) after reacted with biothiols and could selectively detect cysteine (Cys) in dimethyl sulfoxide (DMSO)/H_2O solution (9:1, v/v, 10 mM phosphate buffer saline, pH 3.5) over glutathione (GSH), homocysteine (Hcy) and other analytes with a detection limit of 0.117 μM. In addition, probe **1** responded well to GSH, Hcy and Cys in the same above solution with pH 5.5 and got the detection limits of 0.151 μM, 0.128 μM and 0.037 μM, respectively. Probe **1** was of very low cytotoxicity and successfully applied for imaging of thiols in living cells.

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1. Introduction

Recently, the selective and sensitive fluorescent probes for thiols have increasingly received attention on account of their operation simplicity and capability of imaging intracellular thiols *in vivo* studies. Intracellular biothiols, such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), play momentous roles in cellular growth and redox homeostasis in biological systems through the adjustment between the reduced free thiols and oxidized disulfide forms [1–4]. In general, the abnormal level of cellular thiols is closely related to many diseases. A deficiency of Cys would cause many syndromes, like retarded growth, hair depigmentation, lethargy, liver damage, muscle and fat loss, skin lesions and weakness [5–8]. While an elevated level of Hcy in human plasma is proved to be involved in cardiovascular and Alzheimer's disease [9–11]. And GSH, the most abundant intracellular thiol, serves many cellular functions including xenobiotic metabolism, intracellular signal transduction, and gene regulation [12–17]. Therefore, it is of great scientific and technological interest to recognize and detect special sulfhydryl-containing biomolecules in biochemistry and biomedicine fields.

Over the past several decades, a lot of effective analytical methods have been developed for the detection of thiols in biological systems including high performance liquid chromatography (HPLC) [18], capillary electrophoresis [19], spectrophotometry [20], electrochemical method [21], mass spectrometry (MS) [22], and HPLC–MS [23]. Most of these methods need complicated and costly instruments and troublesome pretreatment procedures such as separation and purification before instrumental analyses. Moreover, few of them are convenient to be applied in intracellular tests due to their limitation in *in vivo* studies. Compared with these, fluorometry has its own advantages with high sensitivity, economy, real-time detection, noninvasiveness and good compatibility with biological samples. As a consequence, numerous fluorescent probes for biothiols based on various mechanisms have been developed [24], including cleavage reaction by thiols [25–34], cyclization reaction with aldehyde [35–43], Michael addition [44–54], metal complexes [55,56], nanomaterials [57–59] and others [60,61].

Rhodamine dyes are widely used as fluorescent probes owing to their high absorption coefficient and broad fluorescence in the visible region of electromagnetic spectrum, high fluorescence quantum yield and photostability [62]. For example, Li group reported a probe for Hg^{2+} based on rhodamine derivative bearing phthalimido Gly [63]. Das and his coworkers reported two rhodamine derivative probes for Hg^{2+} based on through-bond energy transfer (TBET) process [64,65] and a förster resonance energy transfer (FRET) based probe for monitoring pH changes in lipid-dense region of Hct116 cells [66].

Herein, we report a highly selective and sensitive fluorescent probe **1** for quantitative detection of biothiols in living cells. The probe **1** was designed basing on the cleavage of sulfonate ester by mercapto compound (RSH). The compound **2** containing rhodamine B structure is selected as the fluorophore, which has a large stokes shift and long emission wavelength. 2, 4-dinitrophenylsulfonyl moiety is chosen as the PET acceptor, which serves as not only an electrophile but also a quencher of compound **2** (Scheme 1).

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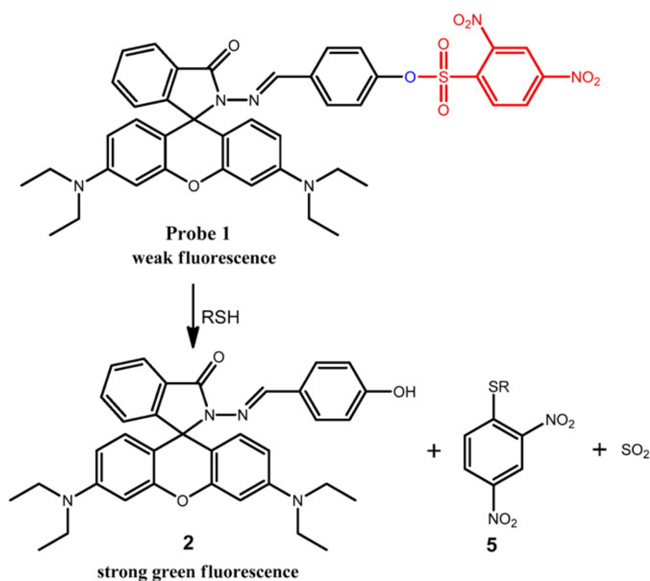

Scheme 1. Proposed sensing mechanism of probe **1** to biothiols.

2. Experimental section

2.1. Materials

Cys and GSH were purchased from Sangon Biotech. Co., LTD. (Shanghai, China). Hcy was obtained from J&K (Beijing, China). 2, 4-Dinitrobenzenesulfonyl chloride was purchased from Alfa Aesar (Tianjin, China). Ultrapure water, which was used throughout the experiments, was obtained from an ALH-6000-U (Aquapro International Company, USA) purification system. HPLC-grade acetonitrile was purchased from Dima Technology (RichmondHill, USA). HeLa cells were obtained from Department of Cell Biology, School of Life Sciences, Lanzhou University (Lanzhou, China). All other chemicals were obtained from qualified reagent suppliers with analytical grade.

2.2. Instruments

Fluorescence spectra were recorded on a Fluorescence spectrophotometer RF-5301pc (SHIMADZU, Japan) with a Xenon lamp and 1.0-cm quartz cells at the slits of 10/10 nm. The fluorescence quantum yields were determined on fluorescence spectrometer FLSP920 (Edinburgh Instruments Ltd., UK). Absorption spectra were measured on a UV–Vis spectrophotometer TU-1810 (PUXI, China). Mass spectra were measured using a mass spectrometer micrOTOF II with ESI mode (Bruker, America). High resolution mass spectra (HRMS) were measured using a spectrometer APEX II 47e FT-ICR with ESI or APCI positive ion mode (Bruker Daltonics, America). NMR spectra were measured using a 400 MHz instrument (JEOL, Japan). The pH values were measured using a digital pH-meter PHSJ-3F (Leici, China). The fluorescence images of cells were taken using a confocal laser scanning microscope TCS SP8 (Leica, Germany) with an objective lens (×40).

2.3. Synthesis

Scheme S1 depicts the synthesis route of probe **1** and compound **2**. Probe **1** was obtained from compound **3** and **4**, both of which were synthesized according to previous reported methods [67,68].

2.3.1. Synthesis of compound 2

Compound **3** (456.0 mg, 1.0 mmol) was dissolved in 10 mL of ethanol, and then added 4-hydroxybenzaldehyde (122.0 mg, 1.0 mmol) into the solution. The stirred mixture was heated to reflux under nitrogen for 6 h and then the solvent was evaporated. The solid was purified by flash column chromatography (CH_2Cl_2/CH_3OH = 100/1) on silica gel, affording the desired compound **2** as an offwhite solid (504.0 mg, yield 90%). m.p. 153–155 °C. ^1H NMR (400 MHz, DMSO-d_6): δ = 9.90 (s, 1H), 8.79 (s, 1H), 7.87 (d, *J* = 6.6 Hz, 1H), 7.61–7.51 (m, 2H), 7.26 (d, *J* = 8.7 Hz, 2H), 7.07 (d, *J* = 7.0 Hz, 1H), 6.72 (d, *J* = 8.6 Hz, 2H), 6.42 (d, *J* = 2.4 Hz, 2H), 6.40 (d, *J* = 8.8 Hz, 2H), 6.32 (dd, *J* = 8.9, 2.4 Hz, 2H), 3.32–3.25 (m, 8H), 1.07 (t, *J* = 7.0 Hz, 12H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 163.89, 160.11, 153.19, 151.63, 149.75, 148.87, 134.03, 129.66, 129.15, 128.17, 126.02, 124.24, 123.33, 116.17, 108.42, 106.19, 97.77, 65.87, 55.45, 44.16, 12.93 ppm. HRMS (ESI, *m/z*) Calcd. for $[C_{35}H_{36}N_4O_3 + H]^+$: 561.2860, found: 561.2865.

2.3.2. Synthesis of probe 1

Compound **3** (456.0 mg, 1.0 mmol) was dissolved in 10 mL ethanol, then added compound **4** (352.0 mg, 1.0 mmol) into the solution. The mixture was stirred and heated to reflux under nitrogen. After 6 h, the reaction mixture was concentrated under reduced pressure to give crude solid, which was purified by silica gel column chromatography using CH_2Cl_2/0–2% methanol as eluent to afford desired brown products (624.0 mg, yield 79%). m.p. 162–164 °C. ^1H NMR (400 MHz, $CDCl_3$) δ = 8.76 (s, 1H), 8.63 (d, *J* = 2.1 Hz, 1H), 8.51–8.32 (m, 1H), 8.07 (d, *J* = 8.7 Hz, 1H), 8.00–7.88 (m, 1H), 7.50 (t, *J* = 8.1 Hz, 4H), 7.12 (d, *J* = 7.1 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 2H), 6.47 (d, *J* = 8.7 Hz, 2H), 6.41 (d, *J* = 2.4 Hz, 2H), 6.23 (dd, *J* = 8.9, 2.4 Hz, 2H), 3.31 (q, *J* = 7.1 Hz, 8H), 1.15 (t, *J* = 7.0 Hz, 12H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.99, 153.20, 151.39, 150.80, 149.21, 148.85, 144.96, 135.58, 134.06, 133.58, 133.32, 129.22, 129.03, 128.42, 128.01, 126.40, 123.97, 123.38, 121.82, 120.27, 107.82, 105.78, 97.67, 66.13, 44.28, 12.56 ppm. HRMS (ESI, *m/z*) Calcd. for $[C_{41}H_{38}N_6O_9 + H]^+$: 791.2494, found: 791.2477.

2.4. General procedure for spectra measurement

The stock solution of probe **1** (1.0 mM) was prepared in DMSO. The analytes (Cys, Hcy, GSH, Phe, Ala, Gly, Glu, Lys, Tyr, Trp, Ser, Asp, Val, Ile, His and CN^-) solutions (8.0 mM) were prepared in deionized water. The test concentration of probe **1** was 10 μM by diluting the stock solution to 4 mL DMSO/H_2O solution (9:1, v/v, 10 mM phosphate buffer saline (PBS)) at various pH. The resulting solution was shaken well at 37 °C for 80 min, and then the fluorescence and UV absorption spectra were recorded. Fluorescence spectra were measured using a fluorescence spectrometer (λ_{ex} = 328 nm, slit: 10/10 nm).

2.5. Cell culture

HeLa cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with heat-inactivated fetal bovine serum (10%), penicillin (100 U/mL), and streptomycin (100 U/mL) at 37 °C in a 95% humidity atmosphere under 5% CO_2 environment.

2.6. Confocal microscope imaging

The cells were seeded in 35 mm diameter glass-bottomed dishes at a density of 3×10^5 cells per dish in RPMI 1640 medium for 24 h. For living cells imaging experiment of probe **1**, cells were incubated with 20 μM probe **1** for 45 min at 37 °C and washed three times with the PBS (pH 5.5 at 37 °C containing 1% DMSO), and then imaged. For *N*-methylmaleimide (NMM, a thiol blocking agent) treated experiments, HeLa cells were pretreated with NMM (1 mM) for 30 min at 37 °C, washed three times with the PBS, and then incubated with 20 μM probe **1** (or incubated with 200 μM of Cys, GSH or Hcy for 30 min prior to addition of probe **1**) for 45 min at 37 °C. Cell imaging was then carried out after washing cells three times with the PBS.

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