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Smart probe for rapid and simultaneous detection and discrimination of hydrogen sulfide, cysteine/homocysteine, and glutathione

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ABSTRACT

Development of a simple probe for simultaneous detection and discrimination of the most important four biothiols (H_2S, Cys, Hcy and GSH) is important but a challenging task. In this paper, a very simple but versatile fluorescent probe was reported, which can be used for simultaneous detection and discrimination of H_2S, Cys/Hcy and GSH. This probe not only can be easily prepared, but also shows rapid (within 150 s), selective and sensitive responses for these four biothiols with distinct fluorescent turn-on signal changes at 465 nm. Moreover, this probe is able to show unique absorbance enhancement at 540 nm for H_2S, and additional fluorescence enhancement at 550 nm only for Cys/Hcy, thus providing a rapid, simultaneous detection and discrimination method for H_2S, Cys/Hcy and GSH. Imaging and discrimination of Cys and GSH in HeLa Cells by this probe were also successfully applied.

1. Introduction

Cysteine (Cys), homocysteine (Hcy), glutathione (GSH) and hydrogen sulfide (H_2S) are the most important small molecular biothiols that play key but diverse roles in biological systems. In addition, concentration levels of these thiols are very useful in the diagnosis of various closely related disease states. For example, abnormal levels of Cys is closely related to diseases of hair depigmentation, slow growth in children, liver damage, edema, loss of muscle and skin lesions [1,2], whereas abnormal levels of GSH correlate with heart problems, liver damage, leucocyte loss, cancer, and aging [3–6], and abnormal levels of H_2S are implicated in central nervous system diseases such as Down syndrome, Parkinson's and Alzheimer's disease [7–9]. Therefore, it is highly important to develop probes for these thiols, and particularly, a single probe that can be used to simultaneously detect and discriminate these thiols would be highly valuable.

Optical probes, especially small molecular fluorescent probes which possess advantages of high sensitivity, low cost, convenience and non-invasiveness, are therefore very attractive for molecule sensing. To date, a large number of fluorescent probes have been developed for sensing thiols [10–15]. However, simultaneous detection and differentiation of Cys, Hcy, GSH and H_2S by a single probe remains a challenge [16]. On the one hand, due to the similar structures and reactivities of Cys, Hcy and GSH, many developed probes only can be used to detect these three thiols as a whole without discrimination [10–12]. On the other hand, although great progress have been achieved recently in developing highly selective probes for H_2S [17–23], Cys [24–28], Hcy [29,30], and GSH [31–33], these probes can only sense one of these biothiols at a time. Very recently, chemists started to develop single probes to meet this challenge, and several elegant probes have been found to be able to simultaneously detect and differentiate Cys/Hcy and GSH [34–44]. However, either of these probes are not responsive for the simplest thiol, H_2S, or have not been reported on the response to H_2S.

Herein, we report a remarkably simple but versatile probe (probe **1** in Scheme 1) that can be used for simultaneous detection and discrimination of H_2S, Cys/Hcy and GSH. This probe has the following merits: (1) it can be easily prepared from readily available inexpensive reagents. (2) It shows rapid (within 150 s), selective and sensitive fluorescent turn-on signal changes for these four thiols over many other common analytes. (3) In addition, it shows additional characteristic optical changes for H_2S in color and Cys/Hcy in fluorescence, respectively, which can be used to discriminate H_2S, Cys/Hcy and GSH simultaneously. To prove its potential utility, we also demonstrated that this probe can be conveniently used for simultaneous detection and discrimination of Cys and GSH in living cells. Considering its readily available and excellent sensing properties, this probe provided a potentially useful tool for biothiol sensing, detection and discrimination.

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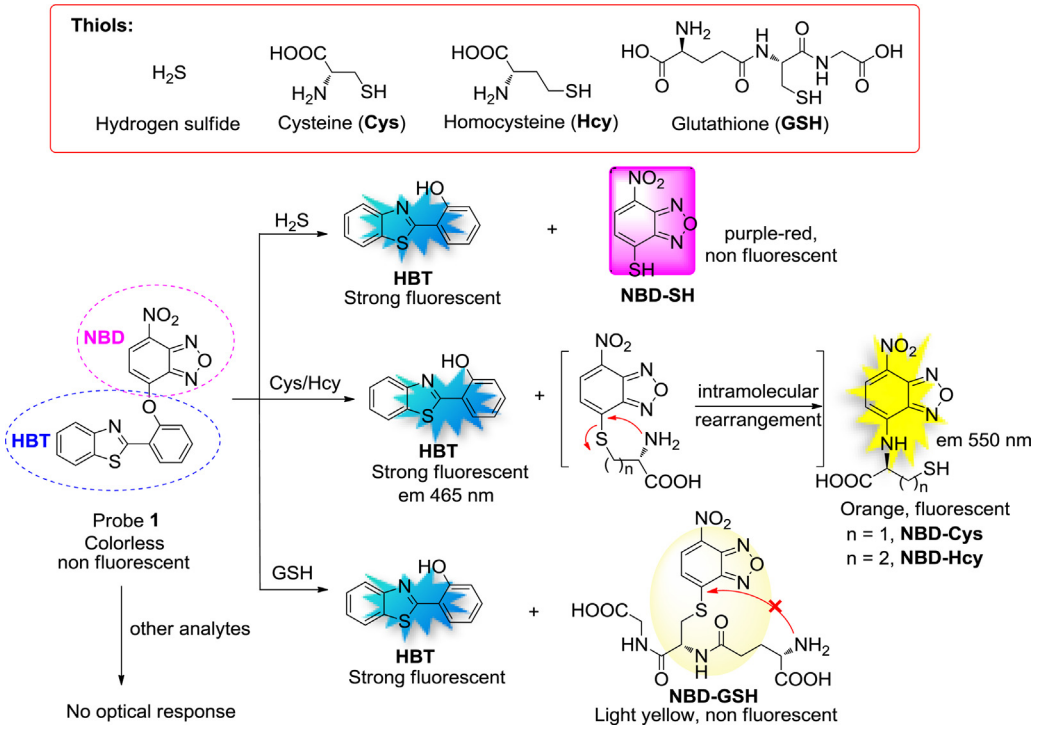

Scheme 1. Structures of H_2S, Cys, Hcy and GSH, and probe **1** for selective detection and simultaneous discrimination of these thiols.

2. Experimental

2.1. Reagents, materials and apparatus

All chemicals were purchased from commercial suppliers and used without further purification except HBT, which was synthesized from 2-aminobenzenethiol and 2-hydroxybenzaldehyde by the published procedures [45]. All solvents were purified prior to use. Distilled water was used after passing through a water ultrapurification system. TLC analysis was performed using precoated silica plates. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 spectrometer, and resonances (δ) are given in parts per million relative to tetramethylsilane (TMS). Coupling constants (J values) are reported in hertz. The low-resolution MS spectra were performed on an electron ionization mass spectrometer. HR-MS data were obtained with an LC/Q-TOF MS spectrometer. UV–vis and fluorescence spectra were recorded at 25 °C on a UV–vis spectrophotometer and a fluorescence spectrophotometer, respectively. The fluorescence quantum yields were determined in PBS buffer (10 mM, pH 7.4, 20% DMSO, v:v) at 25 °C, using quinine sulfate (Φ = 0.58 in 1N H_2SO_4) as standard. Cell imaging was performed in an inverted fluorescence microscopy with a 20× objective lens.

2.2. Synthesis of probe *1*

A solution of 4-chloro-7-nitrobenzofurazan (60 mg, 0.3 mmol), HBT (57 mg, 0.25 mmol) and triethylamine (50 μL) in anhydrous DMF (3 mL) was stirred at room temperature. A dark green precipitate generated gradually in the solution. After 2 h, the solution was poured into ice-water (10 mL) and the precipitate was collected on a filter, washed with CH_3OH and dried under vacuum to afford probe **1** as a pure yellow solid (83 mg, yield 85%). Mp: 219–220 °C. TLC (silica plate): R_f 0.31 (petroleum ether: ethyl acetate 5:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (d, J = 7.8 Hz, 1H), 8.36 (d, J = 8.3 Hz, 1H), 7.92 (d, J = 8.2 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.68 (t, J = 7.1 Hz, 1H), 7.61 (t, J = 7.5 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 7.40 (d, J = 8.0, 1H), 7.36 (d, J = 8.0, 1H), 6.53 (d, J = 8.3 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.19, 152.63, 152.46, 150.84, 145.88, 144.88, 135.61, 135.48, 133.66, 131.54, 130.96, 128.28, 127.22, 126.26, 125.82, 123.41, 123.11, 122.65, 110.86. EI-MS: m/z found 390.20 (M^+). HR-MS: calcd for $C_{19}H_{11}N_4O_4S^+$ ($M+H^+$), 391.0496; found 391.0521. Elemental analysis calcd (%) for $C_{19}H_{10}N_4O_4S$ C 58.46, H 2.58, N 14.35, S 8.21; found C 58.25, H 2.42, N 14.29, S 8.06.

2.3. Optical studies of probe *1* upon addition of various analytes

Stock solutions of probe **1** (1 mM) were prepared in HPLC grade DMSO. Stock solutions (1–10 mM) of the analytes including NaHS, Cys, Hcy, GSH, Gln, Phe, Trp, Ala, leu, Thr, Ser, Asp, Ile, Met, Lys, Gly, Glv, Arg, Tyr, Pyr, His, NaF, NaCl, NaBr, NaI, $Na_2S_2O_7$, Na_2SO_3, Na_2CO_3, Na_2SO_4, $NaHSO_4$, $NaNO_3$, NaSCN, NaAcO, $H_2NCH_2CH_2NH_2$, $HOCH_2CH_2NH_2$, $C_6H_5NH_2$, $C_6H_5CH_2NH_2$, KCl, $MnSO_4$, $FeSO_4$, $MgCl_2$, $CaCl_2$, $HgCl_2$, $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Cu(NO_3)_2$, and $AgNO_3$ were prepared in ultrapure water. ROS/RNS were prepared according our published procedure [46,47]. For optical studies, a solution of probe **1** (10 μM) was prepared in DMSO-PBS buffer solution (1:4, v/v, 10 mM PBS). Then 3.0 mL of the probe **1** solution was placed in a quartz cuvette. The UV–vis or fluorescent spectra were recorded upon addition of analyte of interest at 25 °C with a temperature controller.

2.4. Cell culture and bioimaging

HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (Fetal Bovine Serum), 100 mg/mL penicillin and 100 μg/mL streptomycin in a 5% CO_2, water saturated incubator at 37 °C, and then were seeded in a 12-well culture plate for one night before cell imaging experiments. In the experiment of cell imaging, living cells were incubated with 10 μM of probe **1** (with 0.1% DMSO, v/v) at 37 °C for 30 min and washed with PBS for three times, and then imaged immediately. In the experiment of *N*-ethylmaleimide (NEM) added to the cell