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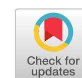
A dual-response fluorescent probe for the discrimination of cysteine from glutathione and homocysteine



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ABSTRACT

A highly selective and sensitive turn-on fluorescent BODIPY-based probe for the simultaneous and selective detection of Cys and Hcy/GSH from dual emission channels was developed. The spatial steric hindrance of the methyl groups at 1- and 7-positions in BODIPY skeleton prevented intramolecular displacement of sulfur with amino group of Hcy but not of Cys. GSH molecular skeleton is larger and amino is far away from sulfydryl group, and the product of the reaction of probe with GSH canstay in thiol phase. Therefore, the probe was successfully applied to the detection of Cys from GSH/Hcy. The confocal microscopy experiments implied that this probe is a promising candidate for imaging of Cys and Hcy/GSH in Hela cells.

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1. Introduction

Biothiols especially low molecular weight thiols such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) play crucial roles in many physiological and pathological processes [1,2]. Cys deficiency is involved in many syndromes, for instance, slow growth in children, hair depigmentation, edema, lethargy, liver damage, loss of muscle, skin lesions and weakness [3]. Elevated level of Hcy is known as a risk factor of Alzheimer's disease, osteoporosis disease, and cardiovascular disease [4]. Glutathione, which is a three peptide compound formed by the junction of glutamic acid, cysteine and glycine, is the most abundant of non-protein thiol (1–10 mM) in cells [5]. The sulfydryl group of GSH can react with free radicals in vivo to protect the body from free radical damage [6,7]. Under oxidative stress, GSH can be converted to its oxidized form (GSSG) to protect cells from oxidative stress through scavenging ROS, and then rapidly revert back to GSH by the action of the enzyme glutathione reductase [8]. Any alteration of the optimum cellular ratios of GSH to GSSG can lead to human pathologies such as heart disease, cancer, strokes and many neurological disorders [9]. Thus, the detection of thiols is highly important for early diagnosis and treatment of diseases, and evaluation of disease progression.

Among the reported detection methods, fluorescent sensing has attracted considerable attention due to its simplicity, low cost, selectivity and sensitivity. A large number of fluorescent probes have been developed in recent years to detect and sense these biologically important species [10–17]. The corresponding design strategies are generally based on the strong nucleophilicity of the thiol group combined with various mechanisms including Michael addition, cyclization reaction, displacement of coordination, cleavage reactions, and others [10–17]. However, albeit these probes can selectively distinguish the biothiols from other amino acids, most of them can not differentiate individual bithiol species from each other due to the similar structures and reactivities of these biothiols. To address this problem, considerable efforts have been devoted to the development of fluorescent probes that are able to respond selectively toward a single biothiol analyte in biological systems. Probes specific for Cys, Hcy or GSH have been constructed successfully [18–32]. Unfortunately, although some advances have been achieved, probes capable of discriminative and simultaneous detection of Cys/Hcy/GSH are still rare and the discrimination between them remains a tough challenge for researchers.

Based on the cyclization of Cys/Hcy with aldehydes or acrylates, pioneered by Strongin's group, the selective detection of Cys/Hcy over GSH could be realized [33–42]. By exploiting the novel thiols-induced S_NAr substitution–rearrangement cascade reaction, the probes could simultaneously and selectively discriminate GSH over Cys/Hcy from different dual emission channels have been reported [43–53]. However, probes capable of discriminating Cys over Hcy/GSH are still infrequent. To the best of our knowledge, only several probes have been reported to date with this capability by taking advantage of more rapid intramolecular displacement of sulfur with amino group of Cys than Hcy and GSH [54–63]. Very recently, our group reported a series of BODIPY dyes containing tetramethyl at 1-, 3-, 5- and 7- positions and N-, O-, or S-aryl substituent at *meso*-position as leaving groups. Two probes were developed for the simultaneous and selective detection of Cys

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and Hcy/GSH from dual emission channels based on the spatial steric hindrance of the methyl groups at 1- and 7-positions in BODIPY skeleton [64]. Herein, we identified that a BODIPY dye containing para-nitrophenyl carboxy group at *meso* position (probe **1** as shown in Scheme 1) was able to differentiate Cys over Hcy/GSH in dual emission channels mode.

2. Experimental

2.1. General Information

All reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Acetonitrile in chromatographic purity and deionized water were used in detection. ^{1}H NMR spectra were recorded on a Varian Model Mercury 400 MHz spectrometer. ^{1}H NMR chemical shifts (δ) are given in ppm (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) downfield from Me_4Si, determined by chloroform (δ = 7.26 ppm). ^{13}C NMR spectra were recorded on a Varian Model Mercury 100 MHz spectrometer. ^{13}C NMR chemical shifts (δ) are reported in ppm with the internal $CDCl_3$ at δ 77.0 as standard, respectively. UV–vis spectra were acquired on a Hitachi U-2900 double beam UV–visible spectrophotometer. Fluorescence spectra were measured by Hitachi F-2500 fluorescence spectrophotometer. Mass spectra were acquired with Agilent 1100 Series LC/MSD, AB SCIEX Triple TOFTM 5600+ mass spectrometer and Bruker Reflex MALDI as matrix (20 kV). All spectra were recorded at 37 °C.

2.2. Synthesis

2.2.1. Synthesis of Bis-(3, 5-dimethyl-1H-pyrrol-2-yl) Methanone (*1a*)

The compound bis-(3, 5-dimethyl-1H-pyrrol-2-yl) methanone (**1a**) was synthesized according to the reported method [63]. 2,4-dimethyl-1H-pyrrole (0.4 mL, 3.9 mmol) and ethyldiisopropylamine (0.64 mL, 3.9 mmol) were dissolved in THF (10 mL) at 0 °C under nitrogen. After adding triphosgene (192 mg, 0.65 mmol, dissolved in 10 mL THF), the solution was stirred for 4 h at 25 °C and monitered by TLC. The reaction mixture was concentrated *in vacuum*, and the residue was separated by column chromatography with 1:1 ethyl acetate/petroleum ether as eluent. The purified product was obtained as a white solid (153.6 mg, 32% yield). ^{1}HNMR (400 MHz, $CDCl_3$, ppm): 8.76 (s, 2H), 5.84 (d, 2H), 2.34 (s, 6H), 2.26 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$, ppm): δ = 175.4, 133.0, 128.0, 127.3, 111.7, 13.0, 12.6.

2.2.2. Synthesis of Probe *1*

To a solution of **1a** (216 mg, 1.0 mmol) in 1,2-dichloroethane (10 mL) was added triethylamine (1.39 mL, 10 mmol), The reaction mixture was stirred at 0 °C for 5 min. Boron trifluoride etherate (1.36 mL, 11 mmol) was added dropwise at 0 °C. Then the mixture was stirred at room temperature for about 4 h until the reaction was complete (monitored by TLC). After adding 4-nitrobenzoyl chloride (222.6 mg, 1.2 mmol) and triethylamine (1.39 mL, 10 mmol), the reaction mixture was stirred at 0 °C for 4 h. The solvent was then removed under reduced pressure and the residue was further purified by column chromatography with 5:1 petroleum/ethyl acetate as eluent. The purified product was obtained as a yellow solid (178 mg, 43% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.42 (s, 4H), 6.05 (s, 2H), 2.55 (s, 6H), 2.10 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 161.75, 156.94, 151.53, 146.97, 139.63, 133.05, 131.53, 126.58, 124.35, 120.83, 14.67, 14.06. HRMS-ESI: Calculated for $[C_{20}H_{19}BF_2N_3O_4]+$: 414.1437; found 414.1431.

2.3. Cell Culture and Imaging

HeLa cells were cultured in Dulbecco modified Eagle medium (DMEM, Gibco) supplemented with 10% fetal bovine serum at 37 °C in 5% CO2. Hela cells were seeded at density of 5×10^4 cells per well (200 μL) in a 6-well plate. For fluorescence imaging, the cells were incubated with probe **1** (10 μM) for 20 min. Then the medium was removed and the cells were washed with PBS buffer two times to remove extracellular compound and the fluorescence images of cells were taken. For the control experiment, the cells were treated with 5 mM *N*-ethylmaleimide (NEM) for 30 min at 37 °C. After washing twice with PBS, the cells were further incubated with 10 μM of probe **1** for 20 min. After washed twice with PBS buffer, the cells were imaged on a confocal microscope.

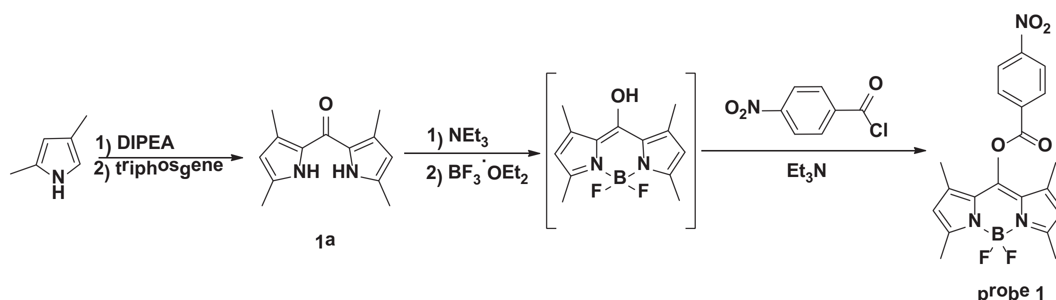
3. Results and Discussion

3.1. Design Concept

Recently, our group reported a series of BODIPY dyes containing tetramethyl at 1-, 3-, 5- and 7-positions and N-, O-, or S-aryl substituent at *meso* position as leaving groups. The probes carrying good leavable group in arylether or aryl thioether linkage at *meso* position with either a-PET or d-PET effect can used to detect Cys and Hcy/GSH from dual emission channels [64]. To explore new probe linked at *meso* position with capability of detection of biothiols by taking advantageous of the dimethyl group with spatial steric hindrance at 1- and 7-positions to prevent intramolecular displacement of sulfur with amino group of Hcy but not of Cys, a *para*-nitrophenyl carboxylate was placed on the *meso* position (probe **1**) was designed and synthesized. The structure of probe **1** was confirmed by ^{1}H NMR and ^{13}C NMR. Based on different thiols-induced S_NAr substitution–rearrangement reaction with Cys and Hcy/GSH, probe **1** could simultaneously and selectively detect Cys and Hcy/GSH.

3.2. Optical Response

The absorption and emission spectra of probe **1** (5 μM) upon addition of 100 μM of Cys and GSH in CH_3CN/PBS buffer (3:7, v/v, 10 mM, pH 7.4) at 37 °C are shown in Fig.1. It is clear that probe **1** itself showed a main absorption at 493 nm, but no fluorescence was observed when excited at 420 nm or 500 nm. Upon the treatment of probe **1** with Cys



Scheme 1. Synthetic route of probe **1**.

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