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A ratiometric fluorescent probe for hydrogen sulfide imaging in living cells

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ABSTRACT

We herein report a turn-on fluorescent probe based on excited state intramolecular proton transfer (ESIPT) mechanism and self-immolative linker for hydrogen sulfide detection. The new probe exhibits high sensitivity and selectivity over other biologically relevant anions. Moreover, we show the utility of the probe for the detection of hydrogen sulfide in living cells as well.

1. Introduction

Hydrogen sulfide (H_2S) is well known as the third signaling molecules in biology along with nitric oxide and carbon monoxide [1]. In mammalian system, the endogenous H_2S is biosynthesized from a cysteine substrate or its derivatives which are catalyzed by several enzymes, such as cystathionine γ-lyase (CSE) [2], cystathionine β-synthase (CBS) [3], cysteine aminotransferase (CAT) [4] and 3-mercaptopyruvate sulfurtransferase (MST) [5]. H_2S is generated in response to regulate energy production in mitochondria of mammalian cells under stress conditions [6]. In addition, H_2S is also involved in various important physiological processes, such as relaxation of vascular smooth muscles, inhibition of apoptosis, intervention of neurotransmission, regulation of inflammation, stimulation of angiogenesis, etc. [7]. Furthermore, the levels of H_2S are associated with many diseases, such as Down syndrome and Alzheimer′s diseases [8]. Despite a number of reports have been published, our knowledge on the significance of hydrogen sulfide in biological system and the mechanism of its action is still far from complete because of the limited availability of detection methods.

There have been several types of probes reported for detecting H_2S, such as colorimetric [9], electrochemical analysis [10], gas chromatography [11] and metal-induced sulfide precipitation [12] often require post-mortem processing and/or destruction of tissues or cell lysates. Among these biological detection technologies, fluorescence spectroscopy is a powerful tool for sensing and imaging trace amounts of samples because of its simplicity, sensitivity, real-time imaging, and especially its nondestructive detection of target biomolecules in live cells or tissues [13–16]. Xian′s group, for example, designed probe containing a thiopyridine moiety to trap H_2S through a nucleophilic substitution reaction [17]. Qian and co-workers reported a ratiometric fluorescent probe using a similar method [18]. On the other hand, Chang′s group and other research groups have developed an azide-based probe, which taps into the reduction of azide group to its parent amine by H_2S [19,20]. He and co-workers developed fluorescent probes based on H_2S-induced tandem chemical reactions [21,22]. Nagano′s group and other groups have employed the displacement strategy to design off–on fluorescent probes for cellular bioimaging [23]. Despite the aforementioned advancement, further development of highly sensitive and selective fluorescent probes for H_2S detection is still intensely sought after because of the critical role of H_2S in physiological and pathological processes.

On the basis of the fact that the *p*-aminobenzyl moiety is able to self-immolate through an intramolecular 1,6-elimination, we reason that importing an H_2S-responsive group to a chromophore could make the chromophore responsive to H_2S. On the other hand, 2-(2′-hydroxyphenyl)-benzothiazole (HBT) was chosen as the chromophore

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as this ESIPT chromophore showed a large Stokes shift, good photostability and corresponding efficient ratiometric fluorescence response. If the self-immolative process was triggered by H_2S so as to release the ESIPT dye of HBT, remarkable ratiometric fluorescence signals would be obtained (Scheme 1). To the best of our knowledge, such a self-immolative linker has never been used to develop a selective fluorescent probe for H_2S previously.

2. Experimental

2.1. Reagents and chemicals

2-aminobenzenethiol, 2-hydroxybenzaldehyde, H_2O_2, HCl and K_2CO_3 were all purchased from Acros. Dulbecco's modified Eagle's medium (DMEM), PBS, fetal bovine serum (FBS), trypsin–EDTA and penicillin/streptomycin were purchased from Invitrogen. Other chemicals were of guaranteed analytical grade and solvents were of HPLC grade and used directly without further purification. Milli-Q water was used throughout all experiments.

2.2. Apparatus

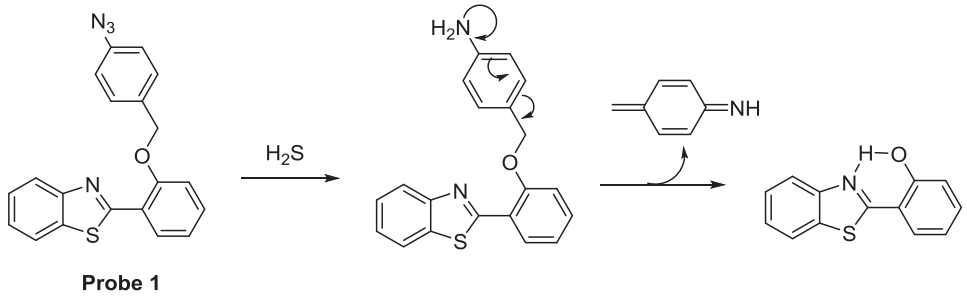
^{1}H NMR and ^{13}C NMR were recorded on a Bruker 400 NMR spectrometer. ESI-MS spectra were measured on a PC Sciex API 150 EX ESI-MS system. Absorption spectra were measured using a Shimadzu UV-1700 spectrophotometer. Fluorescence measurements were carried out with a Shimadzu RF-5301pc spectrofluorophotometer. Cells fluorescence images were acquired using a Leica TCS SP5 Confocal Scanning Microscope. HPLC was performed on an Agilent 1100 HPLC System (column: Agilent C18 5 μM, 4.6 × 250 mm).

2.3. Synthesis of HBT and 1-azido-4-(bromomethyl)benzene

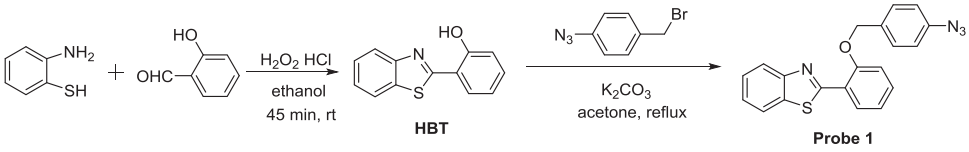
HBT and 1-azido-4-(bromomethyl) benzene were synthesized according to literature [24,25]. The synthetic detail for synthesis of these two compounds is shown in Scheme S1 (Supporting information). Their structures were confirmed by ^{1}H NMR and ^{13}C NMR (Figs. S1–S3, Supporting information).

2.4. Synthesis of probe 1

The probe can be easily obtained through the reaction between HBT and 1-azido-4-(bromomethyl)benzene (Scheme 2). To a stirred mixture of 2-(2-hydroxyphenyl)-benzothiazole (227 mg, 1.0 mmol) and anhydrous potassium carbonate (402 mg, 3 mmol) in 10 mL dry acetone under N_2 atmosphere, was added in a dropwise manner 1-azido-4-(bromomethyl)benzene (233 mg, 1.1 mmol) at room temperature. The resulting reaction mixture was allowed to stir at room temperature overnight. The reaction was quenched with 1 mL of water and the solvent was evaporated under vacuum. The resulting



Scheme 1. Possible H_2S-selective signaling mechanism.



Scheme 2. Synthesis of ratiometric probe 1 for hydrogen sulfide.

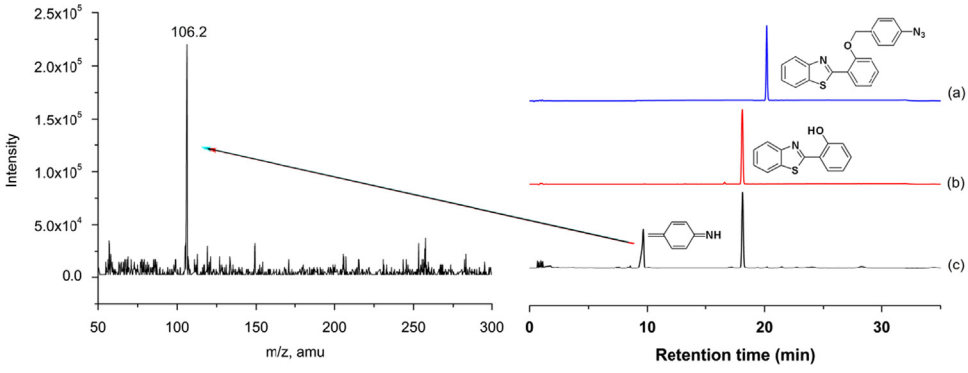


Fig. 1. Right part: HPLC of (a) probe 1 (50 μM), (b) HBT (50 μM) and (c) the reaction product of Probe 1 (50 μM) with NaHS (1 mM) after incubation of them for 1 h. Left part: Mass spectrum of intramolecular 1,6-elimination product.