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Sensors

Capture and Visualization of Hydrogen Sulfide by a Fluorescent Probe**

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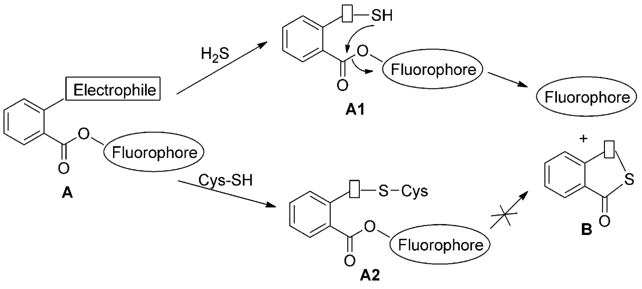
Hydrogen sulfide (H_2S) has been known as a toxic pollutant for many years. However, this molecule has been recently recognized as the third gaseous transmitter (the other two are nitric oxide and carbon monoxide).[1–3] The production of H_2S in mammalian systems has been attributed to at least three endogenous enzymes:[4–7] cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (MPST). These enzymes use cysteine or cysteine derivatives as substrates and convert them into H_2S within different organs and tissues. In addition to these enzymatic pathways, there are also a range of comparably simple chemical events that may liberate H_2S from the intracellular pool of “labile” sulfur, for example, from the “sulfane sulfur” pool (compounds containing sulfur atoms bound only to other sulfur atoms).[8] The production of endogenous H_2S and exogenous administration of H_2S have been demonstrated to exert protective effects in many pathologies. For example, H_2S has been shown to relax vascular smooth muscle, induce vasodilation of isolated blood vessels, and reduce blood pressure. H_2S can also inhibit leukocyte adherence in mesenteric microcirculation during vascular inflammation in rats, suggesting H_2S is a potent anti-inflammatory molecule. Additionally, it has become evident that H_2S is a potent antioxidant and, under chronic conditions, can up-regulate antioxidant defense. Despite the rising interest in H_2S research, fundamental questions regarding regulation of its production, its mechanism of action, and its destruction remain. A critical debate in the field involves the biologically relevant levels of H_2S as current reports varying over 10^5-fold concentration range.[9–12] Obviously, accurate and reliable measurement of H_2S concentrations in biological samples is needed and can provide useful information to understand the function of H_2S. Currently, the major methods for H_2S detection are colorimetric and electrochemical assays, gas chromatography, and sulfide precipitation.[12–16] These methods often require complicated sample processing. Given the high reactivity of H_2S, these methods can yield variable results.[9–12] Fluorescence-based assays could be useful in this field because of their high sensitivity and convenience. However, fluorescence methods for H_2S detection, especially for real-time detection in biological samples, are still very limited so far.[17–19] Herein, we report a reaction-based fluorescent turn-on strategy for the detection of H_2S.

We envisioned that H_2S is a reactive nucleophile in biological systems that can participate in nucleophilic substitution. To detect H_2S selectively, the key is to differentiate H_2S from other biological nucleophiles, especially thiols such as cysteine and glutathione. Theoretically, H_2S can be considered a nonsubstituted thiol. It can undergo nucleophilic reaction twice, whereas other thiols such as cysteine are monosubstituted thiols that can only undergo nucleophilic reaction once. On the basis of this property, we expected that compounds containing bis-electrophilic enters could be useful reagents for H_2S detection. As shown in Scheme 1, H_2S should react with the most electrophilic component of a fluorescent probe such as **A** to form a free SH containing intermediate **A1**. If another electrophile is presented at suitable position, such as the ester group shown in **A1**, the SH group should undergo a spontaneous cyclization to release the fluorophore and form product **B**. This strategy not only can capture H_2S as a stable and analyzable product **B**, but also will allow us to visualize H_2S-related signal through a convenient and sensitive fluorescence measurement. We envisioned that substrate **A** could also react with biological thiols such as cysteine. However, product **A2** should not undergo the cyclization to release the fluorophore. Therefore, the fluorescent signal should be selective only for H_2S.

With this idea in mind, we designed a reactive disulfide-containing probe (compound **1**). This compound was prepared from thiosalicylic acid **2** in two steps using the procedure shown in Scheme 2. The fluorescence property of this probe was tested in aqueous phosphate-buffered saline



Scheme 1. Proposed fluorescent turn-on strategy.

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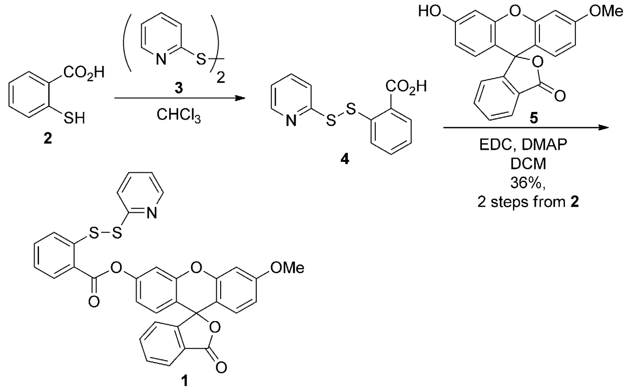
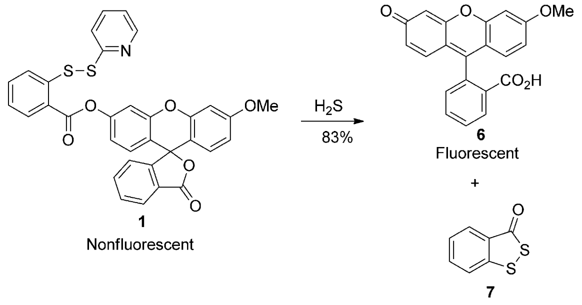


Scheme 2. Synthesis of fluorescent probe **1**. EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DMAP = 4-dimethylaminopyridine; DCM = dichloromethane.

(PBS) solution (pH 7.4). Compound **1** (fluorescence quantum yield: $\Phi = 0.003$) adopted a closed lactone conformation and exhibited no absorption features in the visible region (see the Supporting Information). We found that probe **1** reacted rapidly with H_2S to generate fluorophore **6** ($\Phi = 0.392$) and benzodithiolone **7** in good yields (Scheme 3). In these experiments, NaHS was used as the equivalent of H_2S. It is known that in aqueous state under the physiological pH of 7.4, the major form of H_2S exists as HS^-; the ratio of HS^-/H_2S is approximately 3:1.[9]

As shown in Figure 1, the reaction of **1** with H_2S yielded a significant fluorescence signal. Control experiments using cysteine or glutathione did not lead to any fluorescence increase. As expected, when H_2S and thiols such as glutathione (GSH) co-existed, we still observed strong fluorescence. These results demonstrated that **1** was a selective fluorescent probe for H_2S.



Scheme 3. Fluorescent probes and reaction with H_2S.

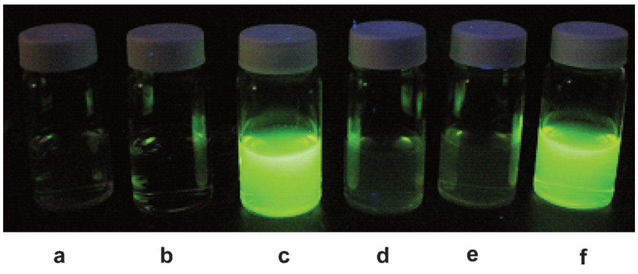


Figure 1. Fluorescent images of probe **1**: a) **1** only (100 μM), b) NaHS only (50 μM), c) **1** (100 μM) + NaHS (50 μM); d) **1** (100 μM) + cysteine (50 μM); e) **1** (100 μM) + glutathione (50 μM), f) **1** (100 μM) + glutathione (50 μM) + NaHS (50 μM), in a mixture of PBS solution (pH 7.4) and CH_3CN (9:1).

The turn-on responses of **1** to H_2S and other biological thiols were also measured by a spectrofluorometer. As indicated in Figure 2, the fluorescence intensity of **1** increased dramatically (55–70-fold) if H_2S was presented in the solution (even when H_2S and other thiols were present together). In addition, the maximum intensity was reached in 1 h, which suggested the reaction was fast.

To demonstrate the efficiency of probe **1** in the measurement of H_2S concentration, **1** was treated with H_2S under a series of different concentrations to obtain a standard curve of emission intensity versus H_2S concentration. The concentration of compound **1** was maintained at 100 μM, while the concentrations of NaHS varied from 0 to 10 μM. As shown in Figure 3, the fluorescent signal was indeed linearly related to the concentration of NaHS in such concentration range. These results demonstrated that probe **1** could detect H_2S both qualitatively and quantitatively.

Next, we used plasma to investigate the potential of probe **1** for use in the detection of H_2S in complex systems. Bovine plasma containing NaHS at different concentrations (0, 50, 100, and 500 μM) were prepared first. These concentrations were within the range of those that have been used to elicit

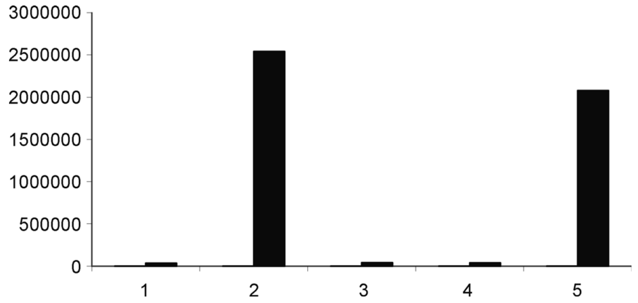


Figure 2. Fluorescence response of probe **1** toward H_2S and other thiols: 1) **1** only (100 μM), 2) **1** (100 μM) + NaHS (50 μM); 3) **1** (100 μM) + cysteine (50 μM); 4) **1** (100 μM) + glutathione (50 μM), 5) **1** (100 μM) + glutathione (50 μM) + NaHS (50 μM); measured in a mixture of PBS buffer (pH 7.4) and CH_3CN (9:1), λ_{ex} 465 nm, 25 °C.

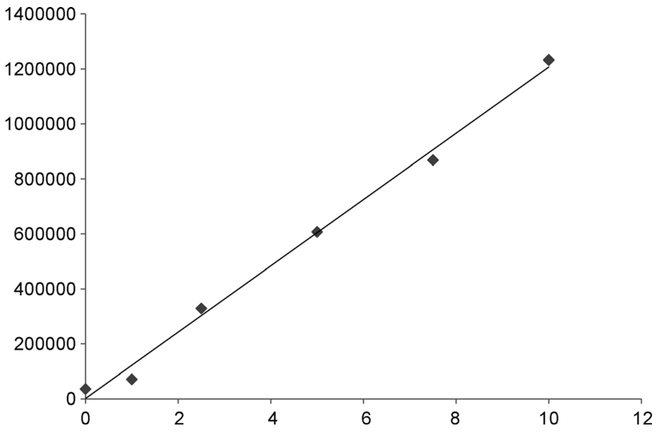


Figure 3. Linear correlation of fluorescent intensity toward H_2S concentration. NaHS concentration: 0, 1, 2.5, 5, 7.5, 10 μM.

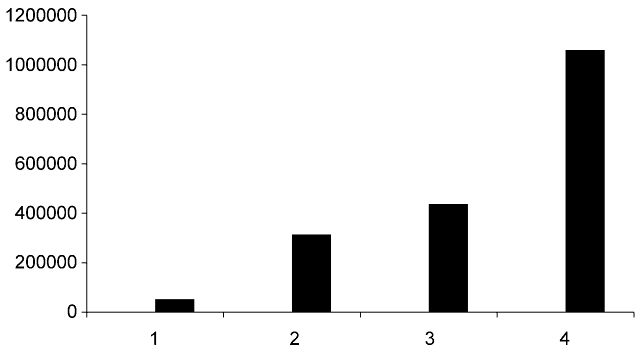


Figure 4. Fluorescence response of probe **1** to H_2S in plasma: 1) probe **1** only, 2) probe **1** + NaHS (50 μM[a], 2 μM[b]), 3) probe **1** + NaHS (100 μM[a], 4 μM[b]), 4) probe **1** + NaHS (500 μM[a], 21 μM[b]). [a] original concentration in plasma, [b] diluted concentration when fluorescence was recorded.

physiological responses of H_2S (10–600 μM).[19–22] These plasma solutions were then diluted and incubated with probe **1**. After 1 h, the mixture was diluted again with PBS solution and fluorescence signals were measured. As expected, strong fluorescence was observed in plasma solutions in the presence of NaHS (Figure 4). We noticed that the fluorescence intensity response to certain H_2S concentration obtained in plasma was lower than the signal obtained in pure buffer solutions. This is likely due to the fact that H_2S can be quickly scavenged by proteins present in plasma.[18] Nevertheless, we conclude that probe **1** can be used for the selective detection of H_2S in complex biological systems such as plasma.

We also used cultured COS7 cells to investigate the potential of **1** for use in the detection of H_2S in cells. As shown in Figure 5, COS7 cells were incubated with compound **1** (100 μM) for 30 min, and we did not observe any fluorescent cells. Strong fluorescence in the cells was induced after treatment with sodium sulfide (250 μM). Thus, we conclude that probe **1** can be used for the detection of H_2S in cultured cells.

In summary, we reported in this study a H_2S-mediated benzodithiolone formation under mild conditions. This reaction proved to be selective for H_2S and it did not proceed with other biological thiols such as cysteine and glutathione. Based on this reaction, a fluorescent probe, that is, compound **1**, was developed for the detection of H_2S. The efficiency of this probe was demonstrated in aqueous buffers and plasma, as well as in cells. Using this strategy, the concentration of H_2S can not only be measured by the fluorescence signal, but also be assessed from the analysis of the benzodithiolone product. We are now actively pursuing more specific H_2S fluorescent probes based on this new benzodithiolone formation and related reactions.

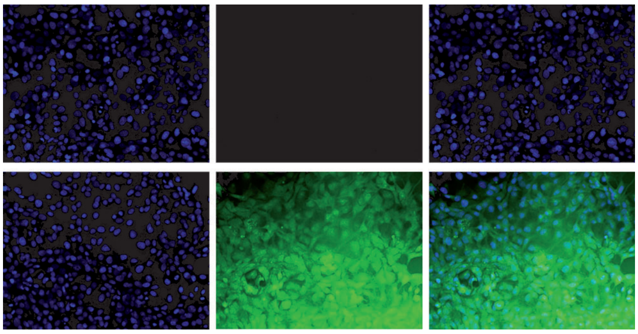


Figure 5. Fluorescence images of H_2S detection in COS7 cells using probe **1**. COS7 cells on glass coverslips were incubated with **1** (100 μM) for 30 min, and then subjected to different treatments. Top row: control (no sodium sulfide was added); bottom row: cells treated with sodium sulfide (250 μM).

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