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A highly sensitive fluorescent probe for imaging hydrogen sulfide in living cells

Huatang Zhang, Ping Wang, Ganchao Chen, Hon-Yeung Cheung, Hongyan Sun *

Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong, PR China

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ABSTRACT

We report herein a fluorescent probe for the detection of hydrogen sulfide (H_2S) with high sensitivity and selectivity. The probe is installed with double azide groups and displays high sensitivity to H_2S (around 120-fold turn-on response). It reacts with H_2S with high selectivity over other reactive sulfur, nitrogen, and oxygen species. The probe was also applied to detect H_2S in living cells.

Hydrogen sulfide (H_2S), commonly perceived as a poisonous gas with an unpleasant smell, has recently been found as an important gaseous signaling molecule in mammalian systems just like NO and CO.[1] A number of endogenous enzymes, including cystathionine β-synthase (CBS), cystathionine γ-lyase, and 3-mercaptopyruvate sulfurtransferase, are responsible for converting sulfur-containing molecules into H_2S.[2] H_2S is involved in regulating a diverse spectrum of important physiological processes, such as relaxation of vascular smooth muscles, inhibition of apoptosis, intervention of neurotransmission, regulation of inflammation, stimulation of angiogenesis, etc.[3] The concentration of endogenous H_2S can vary from nano to millimolar levels across different physiological and pathological states.[4] H_2S is known to play a protective role under physiological concentrations. Abnormal levels of H_2S, however, is associated with several diseases, including Alzheimer's disease, Down syndrome, diabetes, and liver cirrhosis.[5] Despite the widely recognized role of H_2S in the regulation of numerous physiological processes, our knowledge on the production, tissue specificity, and mechanism of action of H_2S is still far from complete. It therefore presents significant research value to develop novel methods for accurate and sensitive H_2S detection to further understand its biological roles and molecular mechanism.

Several methods have been traditionally employed for H_2S detection, namely colorimetry,[6] electrochemical assay,[7] gas chromatography, and sulfide precipitation.[8] These methods, however, are destructive and require tedious preparation sequence. Fluorescence-based methods have recently emerged as a highly desirable and sensitive approach for H_2S detection. The most notable advantage of this method is that it allows real-time monitoring of the biological samples. Due to this prominent feature, it has spurred intensive research efforts to design fluorescence-based probes for H_2S detection. Chang's group and other research groups, for example, have developed an azide-based probe, which taps into the reduction of azide to its parent amine by H_2S.[9] Xian and Qian, on the other hand, designed probes containing a thiopyridine moiety to trap H_2S.[10] The S-SH moiety formed by the nucleophilic attack of H_2S can then undergo cyclization and release fluorophore. Nagano and co-workers designed a fluorescent probe for cellular bioimaging based on azamacrocyclic Cu^{2+} complex chemistry.[11] He and others developed fluorescent probes based on H_2S-induced tandem chemical reactions.[12] 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.

Herein we report a fluorescent probe **1** installed with double azide groups to detect intracellular H_2S (Scheme 1). Our experiment showed that the probe generated more than 100-fold turn-on response with treatment of H_2S. We therefore ranked it as a highly sensitive fluorescent probe for H_2S.

Probe **1** can be conveniently prepared from 5(6)-carboxy-rhodamine (Scheme 2). 5(6)-Carboxy-rhodamine was synthesized based on reported procedure.[13] The amine group of 5(6)-carboxy-rhodamine was converted to the azide group by the Sandmeyer reaction with a yield of 36%. The structural characterization of

* Corresponding author. Tel.: +852 3442 9537; fax: +852 3442 0522.
E-mail address: hongysun@cityu.edu.hk (H. Sun).

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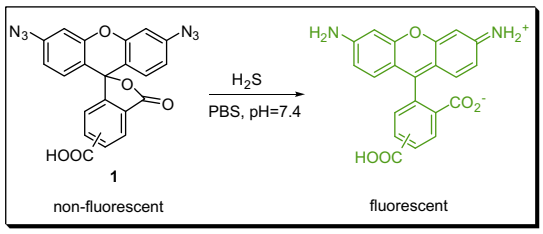
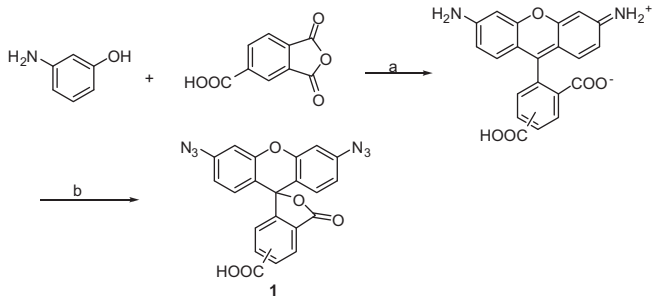


Scheme 1. Structure of probe **1** and the proposed 'turn-on' mechanism of probe **1** for H_2S detection.



Scheme 2. Synthetic scheme of probe **1**. Reagents and conditions: (A) H_2SO_4, 180 °C, 6 h, 27%; (B) HCl/H_2O, $NaNO_2$, NaN_3, rt, 4 h, 36%.

probe **1** was confirmed by ^1H NMR, ^{13}C NMR, IR, and HR-MS (ESI). Though it is well known that aryl azides are subject to photolysis under UV light,[14] probe **1** based on rhodamine is excited under visible light and should be photostable for biological applications.

Following chemical synthesis and characterization, we tested the fluorescence response of the reaction between **1** and H_2S (using Na_2S as an equivalent). Probe **1** did not display noticeable fluorescence in PBS buffer (20 mM, pH 7.4). However, a greenish solution was observed with the naked eye within minutes after mixing probe **1** and H_2S in PBS buffer (Supplementary data, Fig. S1). Inspired by this observation, we performed fluorescence titration of probe **1** and Na_2S of various concentrations (0.5–500 μM). The results indicated that the fluorescence intensity increases with increasing concentrations of Na_2S (Fig. 1). A strong emission peak at 525 nm was detected when the reaction mixture was excited at 480 nm. Around 120-fold fluorescence enhancement was obtained when the reaction was complete, underscoring the high sensitivity of the reaction. Further data analysis also revealed an excellent linear relationship ($R^2 = 0.9924$) between the fluorescence signal at 525 nm and the concentration of Na_2S. The detection limit is determined to be 1.12×10^{-7} M by setting the signal-to-noise (S/N) ratio to 3:1.[15] Taken together, these experiments clearly demonstrated that our probe can be used to detect H_2S both with high sensitivity and in a quantitative manner.

The reaction kinetics of probe **1** and H_2S is an important parameter to examine its biological applicability on account of the rapid catabolism of H_2S under physiological conditions. To obtain the reaction kinetics, we incubated the probe with Na_2S at 37 °C in PBS buffer (pH 7.4), and the fluorescence intensity was recorded at different time points. The fluorescence signal at 525 nm was plotted as a function of time for data analysis. As shown in Figure 2A, the fluorescence signal increases rapidly at the beginning and reached steady state at around 45 min. The pseudo-first-order rate, k_{obs}, was found to be 2.15×10^{-3} S^{-1} by fitting the fluorescence intensity data into the following equation: $\mathrm{Ln}\,(F_{max} - F_t) = -\mathrm{Ln}F_{max} - k_{obs} \times t$. The reaction rate ($k_2$) was calculated as 4.30 M^{-1} S^{-1}, which is comparable with other H_2S probes.[9]

A major challenge of H_2S detection in biological systems is to develop a probe that exhibits notably distinctive response to H_2S over other cellular molecules.[12] The functional behavior of a practical H_2S probe should not be interfered by other biomolecules present in the system. To investigate the selectivity of probe **1**, various biologically relevant species were incubated with probe **1** in PBS buffer to test their fluorescence response (Fig. 2B). These biologically relevant species include reactive sulfur species (homocysteine, glutathione, cysteine, and mercaptoethanol, SO_3^{2-}, $S_2O_3^{2-}$, $S_2O_4^{2-}$, $S_2O_5^{2-}$), reactive oxygen species (H_2O_2, HOCl), reactive nitrogen species (NO_2^-, NO_3^-), and other anions (F^-, Cl^-, Br^-, I^-, etc.). Among all the tested molecules, only homocysteine, glutathione, cysteine, and $S_2O_3^{2-}$ showed limited fluorescence response. The fluorescence increment of these molecules, however, is far below that of Na_2S. The highest increase of fluorescence was captured with the cysteine sample (3.7-fold), and the increment was produced with a cysteine solution at twice the concentration of the Na_2S solution. The selectivity of probe **1** toward H_2S over cysteine,

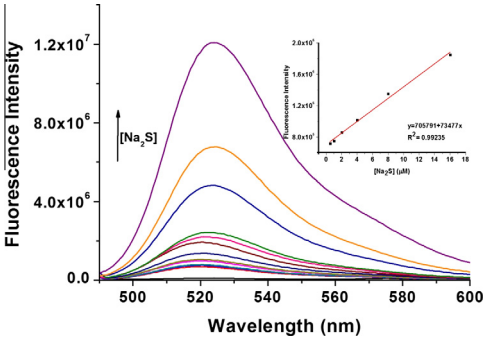


Figure 1. Fluorescence emission spectra of probe **1** (5 μM) in PBS buffer (pH = 7.4) with addition of increasing concentrations of Na_2S ([Na_2S] = 0, 0.5, 1.0, 2.0, 4.0, 8.0, 16, 32, 62.5, 125, 250, and 500 μM, λ_{Ex} = 480 nm, λ_{Em} = 490–600 nm). The inset figure shows that the fluorescence signal increases linearly with increasing concentrations of Na_2S.

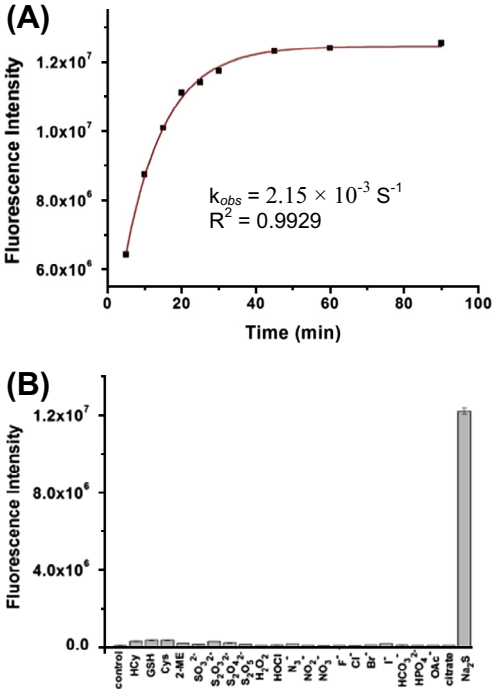


Figure 2. (A) Time course experiment of probe **1** (5 μM) reacting with Na_2S in PBS buffer (pH = 7.4) at 37 °C. (B) Selectivity experiments of probe **1** (5 μM) with 21 different reactive sulfur, nitrogen, and oxygen species (1 mM) and Na_2S (500 μM) in PBS buffer (pH = 7.4). (λ_{Ex} = 480 nm, λ_{Em} = 525 nm.)