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Reaction-based colorimetric and fluorogenic signaling of hydrogen sulfide using a 7-nitro-2,1,3-benzoxadiazole–coumarin conjugate

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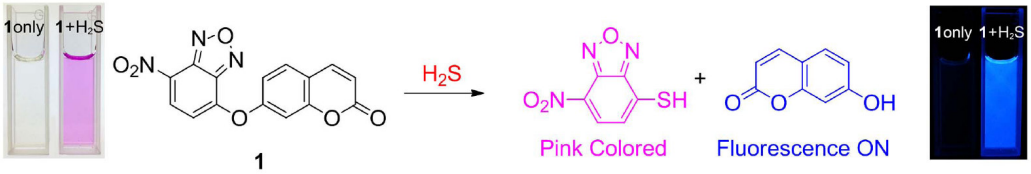

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ABSTRACT

A novel reaction-based probe for the dual signaling of hydrogen sulfide (H_2S) was investigated. The selective H_2S-induced cleavage of the ether linkage of the 7-nitro-2,1,3-benzoxadiazole (NBD) and 7-hydroxycoumarin conjugate resulted in a dual signaling behavior. The colorimetric and fluorogenic signaling behaviors were attributed to the H_2S-induced generation of 7-nitrobenzo-1,2,5-oxadiazole-4-thiol (NBD-SH) and 7-hydroxycoumarin, respectively. The signaling behavior was analyzed by ratiometry. The selective signaling of H_2S over other common metal ions and anions was possible with a detection limit of 1.6×10^{-6} M in an aqueous DMSO solution.

Selective sensing of important chemical species that play essential roles in chemical, biological, and industrial processes is one of the most important research topics.[1] Among many important analytical targets, hydrogen sulfide (H_2S) or its ionized form, sulfide ion (S^{2-}), has attracted considerable interest because of their importance in various aspects of chemical, biological, and environmental sciences.[2] H_2S is a toxic gas and is generated by bacterial degradation of organic matter, extraction of natural gas or oil, mining activity, and refining of natural gas and crude oil.[3] The main industrial sources of H_2S are kraft-pulp mills, petroleum refineries, gasification of coal, and sewage treatment plants.[4]

Despite its toxicity, H_2S is an important industrial material for the production of elemental sulfur, metal sulfides, and thioorganic compounds such as thiols and sulfides.[5] In addition, H_2S has been widely employed as an analytical reagent and in the manufacture of heavy water.[6] Particularly, the application of H_2S in the preparation of nanostructured metal sulfides has recently become increasingly important because of their applications in a variety of devices such as solar cells, light-emitting diodes, sensors, lithium-ion batteries, and fuel cells.[7]

The ability to detect H_2S in low concentrations is extremely important because of its effects on humans. Long-term low-level exposure to H_2S may result in fatigue, loss of appetite, headache, irritability, poor memory, and dizziness.[8] In fact, H_2S gas stimulates the central nervous system, causing hyperpnoea that leads to apnoea, convulsions, unconsciousness, and death.[9] According to the National Institute of Occupational Safety and Health (NIOSH), the concentration of H_2S immediately dangerous to life or health (IDLH) is 100 ppm, and the recommended exposure limit is 10 ppm for a maximum duration of 10 min.[10] Hence, it is of great importance to develop simple and sensitive methods for the determination of H_2S in various samples at low concentrations.

Analytical techniques for the detection of H_2S have been recently reviewed.[11] H_2S analysis has been most frequently carried out using gas chromatography,[12] atomic absorption spectrometry,[13] and electrochemical techniques.[14] Recently, reaction-based optical chemosignaling systems for H_2S have attracted considerable research interest.[15,16] For example, the H_2S-assisted reductions of azide derivatives in rhodamine,[17] dansyl,[18] naphthalimide,[19] benzothiazolyl naphthalene,[20] and near-infrared cyanine dye[21] to the corresponding amines have been successfully used as selective switches for the signaling of H_2S. The reductions of the nitro groups of nitrophenol-substituted heptamethine cyanine and nitroolefin-based coumarin have also been used for this purpose.[22,23] Other useful methods employed for the selective H_2S signaling include the reduction of selenoxide derivative of the boron-dipyrromethene (BODIPY) dye[24] and the nucleophilic cleavage of fluorescein–dinitrobenzenesulfonate[25] and fluorescein ester with an adjacent disulfide bond.[26] Moreover, the high affinity of S^{2-} ions to Cu^{2+} ions in the Cu^{2+} complex of fluoresceins conjugated to an azamacrocyclic or dipicolyl chelating subunit[27,28] has also been utilized for the purpose.

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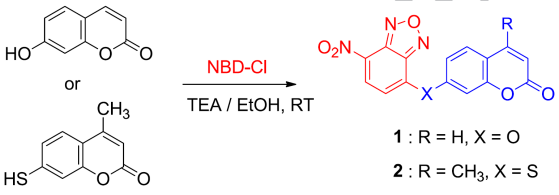
The biological imaging of H_2S in the living cells has attracted much research interest because it is one of the most important endogenously generated gaseous signaling compounds; it is known to be involved in a variety of physiological processes.[29] However, studies to monitor H_2S in chemical and environmental analytes have been much less investigated. Recently, a novel colorimetric signaling method has been developed by using the H_2S-assisted cleavage of ether, thioether, and selenoether groups of the NBD-functionalized derivatives that exhibit colorimetric signaling behavior.[30] Herein, we designed a novel NBD–fluorophore conjugate containing a fluorescent coumarin subunit that could furnish fluorogenic signaling behavior because of the H_2S-assisted generation of a fluorophore, in addition to the colorimetric behavior.[31]

NBD–hydroxycoumarin conjugate **1** was prepared by the reaction of 7-hydroxycoumarin with 4-chloro-7-nitro-1,2,3-benzoxadiazole (NBD-Cl) (TEA, EtOH, 72%, Scheme 1). For comparison purposes, closely related mercapto analogue **2** was also prepared by the reaction of 7-mercapto-4-methylcoumarin with NBD-Cl (TEA, EtOH, 86%, Scheme 1). Compound **1** exhibited an absorption band at 376 nm in the UV− vis spectrum and yielded a colorless solution in 50% aqueous DMSO buffered at pH 7.1 using hydroxyethyl-piperazineethane sulfonic acid (HEPES). A strong absorption band at 551 nm appeared on treating **1** with 10 equiv H_2S,[32] along with a concomitant color change from colorless to pink (the inset of Fig. 1). Other anions did not induce any noticeable change in the UV–vis spectrum as well as the color of the solution. A large change in the wavelength at the maximum absorbance ($\Delta\lambda$ = 175 nm) was induced by the signaling; therefore, a ratiometric analysis was performed. Figure 1 shows that the absorbance ratio at the two characteristic wavelengths of 551 and 376 nm, A_{551}/A_{376}, changed over 200-fold for H_2S. On the other hand, the ratios for the rest of the surveyed anions were relatively constant.



Scheme 1. Preparation of NBD–coumarin conjugates **1** and **2** for H_2S signaling.

Probe **1** showed a weak fluorescence in 50% aqueous DMSO solution buffered at pH 7.1 (HEPES buffer). In the presence of H_2S, an intense emission band at 454 nm was observed (the inset of Fig. 2). The fluorescence enhancement estimated by the fluorescence intensity ratio of **1** at 454 nm in the presence and absence of added anions, I/I_o, for H_2S was 17.42 (Fig. 2). An intense blue fluorescence was observed under UV–lamp illumination. The fluorescence was attributed to 7-hydroxycoumarin that was produced by the H_2S-induced cleavage reaction of **1**. The responses of **1** towards other anions were negligible except for the azide ions (I/I_o = 2.27). The values of I/I_o of **1** for the rest of the anions were nearly constant, ranging between 1.06 for F^- and 1.25 for SO_4^{2-} anions.

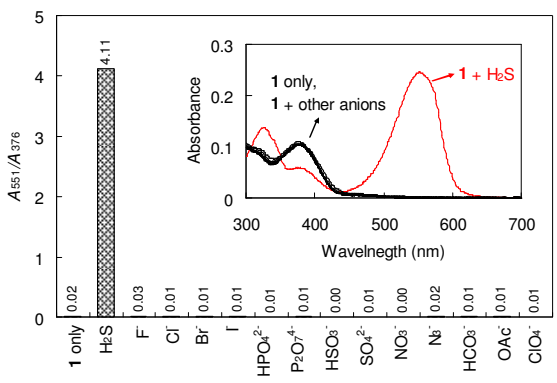


Figure 1. Absorbance ratio at 551 and 376 nm (A_{551}/A_{376}) of **1** in the presence of common anions. Inset: changes in UV–vis spectrum. [**1**] = 1.0 × 10^{-5} M, [H_2S] = [A^{n-}] = 1.0 × 10^{-4} M in a mixture of HEPES buffer (pH = 7.1) and aqueous DMSO (1:1, v/v). [HEPES buffer] = 10 mM.

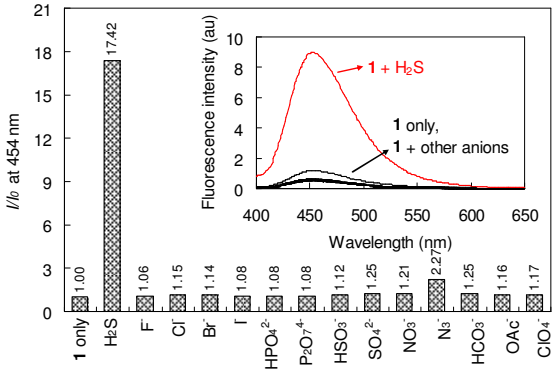
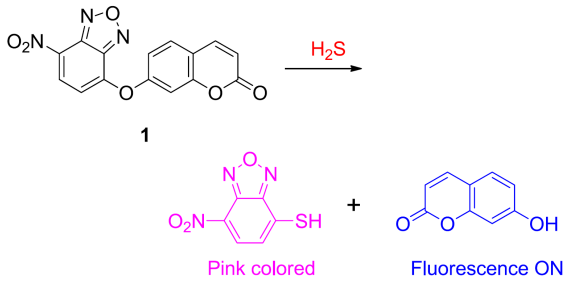


Figure 2. Fluorescence intensity ratio I/I_o of **1** at 454 nm in the presence of common anions. Inset: changes in fluorescence spectrum. [**1**] = 5.0 × 10^{-6} M, [H_2S] = [A^{n-}] = 5.0 × 10^{-5} M in a mixture of HEPES buffer (pH = 7.1) and aqueous DMSO (1:1, v/v). [HEPES buffer] = 10 mM. λ_{ex} = 350 nm.

The signaling behavior was observed because of the H_2S-induced cleavage of the ether group of **1** to yield 7-nitrobenzo-1,2,5-oxadiazole-4-thiol (NBD-SH) and 7-hydroxycoumarin (Scheme 2). Montoya et al. employed the cleavage reaction of bridging thioether group of a NBD-thioether for selective H_2S signaling.[30a] We also reported H_2S signaling by the cleavage reaction of selenoether, thioether, and ether derivatives with similar structure.[30b] After the cleavage of **1**, the resulting NBD-SH product showed chromogenic signaling behavior (the color changed from colorless to pink), whereas the 7-hydroxycoumarin product exhibited fluorogenic (off–on type) signaling behavior. Often, the NBD derivatives with substituents containing bridging O, N, or S atom at the 4 position showed very weak emission;[33] therefore, fluorogenic signaling could not be obtained with produced NBD-SH. We designed a dual signaling probe by conjugating NBD subunit with a fluorophore to obtain the desired fluorogenic behavior along with chromogenic signaling. The signaling process could be followed by ^{1}H NMR and mass spectral measurements. The ^{1}H NMR spectrum of the purified product of the cleavage reaction of probe **1** with 2 equiv H_2S revealed the characteristic NMR peaks of NBD-SH and 7-hydroxycoumarin (Fig. 3). The transformation of **1** to NBD-SH and 7-hydroxycoumarin was also confirmed by TLC experiments (R_f = 0.71, 0.49, and 0.14 for probe **1**, 7-hydroxycoumarin, and NBD-SH, respectively, in CH_2Cl_2:CH_3OH = 9:1, v/v). Moreover,

the mass spectrum of the reaction mixture of **1**–H_2S system showed a prominent peak for 7-hydroxycoumarin at m/z = 162.03.



Scheme 2. H_2S signaling by NBD–coumarin conjugate **1**.

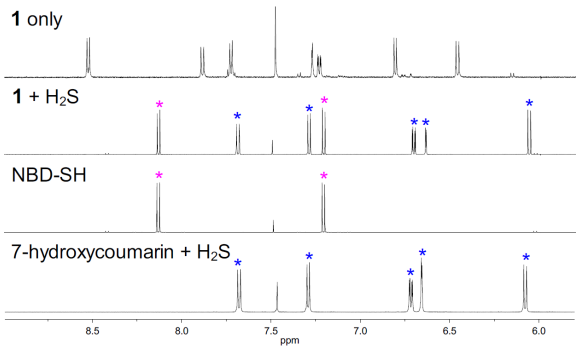


Figure 3. Partial ^{1}H NMR spectra of **1**, the reaction product of **1** with H_2S, NBD-SH, and 7-hydroxycoumarin. [**1**] = [NBD-SH] = [7-hydroxycoumarin] = 1.0 × 10^{-2} M in $CDCl_3$:CD_3OD = (3:2, v/v). NBD-SH was independently prepared by following the reported procedure.[30a]

In order to check the general applicability of the probe design using an NBD–fluorophore conjugate, the signaling behavior of the NBD–mercaptocoumarin analogue **2** was tested. We expected that the oxygen-to-sulfur exchange could provide further information on the H_2S signaling of the NBD–fluorophore conjugates, such as selectivity and signaling speed. The mercaptocoumarin fluorophore, which is frequently employed as a subunit of the fluorescent signaling probes, was selected with the assumption that the signaling behavior would be enhanced by affecting the cleavage process of the bridging thioether group. Compound **2** also showed prominent colorimetric signaling behavior toward H_2S because of the resulting chromogenic NBD-SH product (Fig. S1, Supplementary data). However, the expected fluorescence off–on type signaling behavior was not observed because of the low fluorescence intensity of the resulting 7-mercaptocoumarin product under the measuring conditions (Fig. S2, Supplementary data).[34]

The efficient and selective fluorescence signaling of H_2S by **1** was observed between pH 4.8 and 7.1 (Fig. S3, Supplementary data). With an increase in the pH of the solution, the response related to the maximum absorbance of the resulting NBD-SH product at 551 nm, increased steadily up to pH 4.8 and then became constant. This may be attributed to the formation of the deprotonated thiolate form (NBD-S^-) of the NBD-SH product (pK_a of NBD-SH = 2.6).[30a] On the other hand, the absorbance at 376 nm attributed to the absorption of the 7-hydroxycoumarin product slightly decreased up to pH 7 and then significantly increased beyond pH 8. This is due to the deprotonation of the phenolic hydroxyl group of the 7-hydroxycoumarin chromophore (pK_a = 7.11).[35] A relatively optimized signaling speed was observed in 50% aqueous DMSO buffered at pH 7.1 (HEPES buffer). The H_2S signaling was accomplished within 2 min (Fig. S4, Supplementary data), whereas probe **1** showed no discernible responses up to 3 h after the sample preparation.

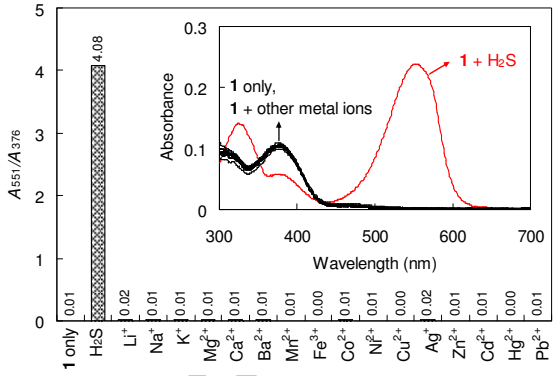


Figure 4. Absorbance ratio of **1** at 551 and 376 nm (A_{551}/A_{376}) in the presence of common metal ions. Inset: changes in UV–vis spectrum. [**1**] = 1.0 × 10^{-5} M, [H_2S] = [M^{n+}] = 1.0 × 10^{-4} M in a mixture of HEPES buffer (pH = 7.1) and DMSO (1:1, v/v). [HEPES buffer] = 10 mM.

Fortunately, probe **1** revealed no significant responses toward common metal ions (e.g., alkali, alkaline earth, and transition metal ions) under the measuring conditions (Fig. 4 and S5, Supplementary data). Most of the metal ions showed nearly constant absorbance ratio (A_{551}/A_{376}) less than 0.02. This additional advantage makes **1** suitable for application in the selective H_2S signaling of environmental samples. The possible interference from other sulfur containing anions and biological species such as thiosulfate, cysteine, and glutathione was also tested. The response from sulfur containing anions HSO_3^-, $S_2O_3^{2-}$, and $S_2O_4^{2-}$ was not prominent. On the other hand, biological thiol species of cysteine, homocysteine and glutathione showed considerable responses at 420 or 475 nm. However, the ratiometric treatment of the responses using the absorbance ratio of **1** at 551 and 376 nm (A_{551}/A_{376}) showed that the selective signaling of H_2S was not significantly affected by these sulfur containing anions and biological thiol species (Fig. S6, Supplementary data).

The competitive signaling of H_2S by **1** was investigated under identical measuring conditions. The H_2S-selective signaling of **1** was not significantly affected by the presence of common anions in the background (Fig. 5 and S7, Supplementary data). In fact, the absorbance ratio of the H_2S signaling of **1** in the presence and absence of competing anions at A_{551}/A_{376} varied in a limited range of 4.17–4.44. The results further demonstrated that NBD–hydroxycoumarin conjugate **1** could be used for H_2S detection in real samples.

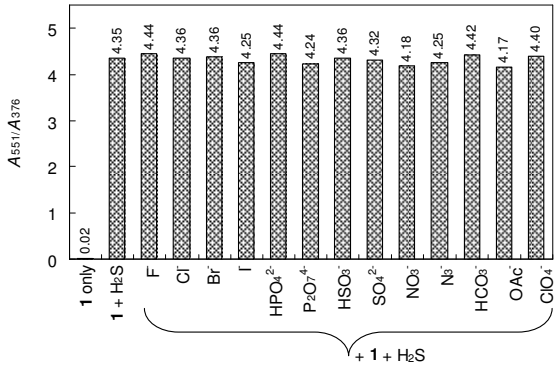


Figure 5. Competitive signaling of H_2S by **1** in the presence of various anions in the background. [**1**] = 1.0 × 10^{-5} M, [H_2S] = [A^{n-}] = 1.0 × 10^{-4} M in a mixture of HEPES buffer (pH = 7.1) and DMSO (1:1, v/v). [HEPES buffer] = 10 mM.

Finally, the quantitative H_2S signaling behavior of **1** was investigated by UV–vis titration. With the increase in the H_2S concentration, the absorbance of **1** at 551 nm increased steadily, while the absorbance at 376 nm decreased (Fig. 6). The changes in the absorbance of **1**, as a function of the H_2S concentration, could be readily analyzed by ratiometry using the ratio of absorbance obtained at the two characteristic wavelengths, 551 and 376 nm. The ratio changed significantly up to 5.5 equiv (5.5 × 10^{-5} M) of the H_2S concentration and could be used as a calibration plot for the quantification of H_2S. From this concentration-dependent plot, the detection limit of **1** for the determination of H_2S was estimated to be 1.6 × 10^{-6} M.[36] The calibration curve obtained from fluorescence titration was also useful for the quantitative analysis of H_2S up to 4.0 × 10^{-5} M with a detection limit of 2.7 × 10^{-6} M (Fig. S8, Supplementary data).

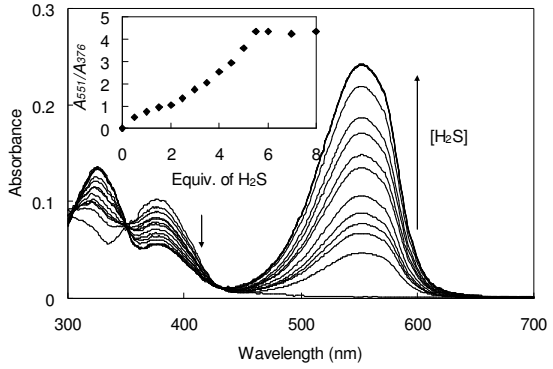


Figure 6. Changes in the UV–vis spectrum of **1** as a function of H_2S concentration. Inset: Changes in absorbance ratio (A_{551}/A_{376}) as a function of H_2S concentration. [**1**] = 1.0 × 10^{-5} M, [H_2S] = 0–8 × 10^{-5} M in a mixture of HEPES buffer (pH = 7.1) and DMSO (1:1, v/v). [HEPES buffer] = 10 mM.

In summary, a novel reaction-based probe for H_2S signaling using an H_2S-assisted cleavage process was investigated. The cleavage of the NBD–hydroxycoumarin conjugate by H_2S resulted in the pronounced colorimetric and fluorogenic signaling of H_2S. After the cleavage reaction, the resulting NBD-SH chromophore and 7-hydroxycoumarin fluorophore products showed colorimetric and fluorogenic behaviors, respectively. The designed reaction-based strategy using a selectively cleavable chromophore and fluorophore conjugate could be useful for the development of other smart molecular devices.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:****.

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31. During the reviewing process of this manuscript, another paper concerning the fluorescence signaling of hydrogen sulfide using closely related NBD-fluorescein conjugate (Wei, C.; Zhu, Q.; Liu, W.; Chen, W.; Xi, Z.; Yi, L. *Org. Biomol. Chem.* **2014**, *12*, 479–485; Title: NBD-based colorimetric and fluorescent turn-on probes for hydrogen sulfide) was published.
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