Fluorescent Probes

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# Fluorescent Probes for the Detection of Hydrogen Sulfide in Biological Systems**

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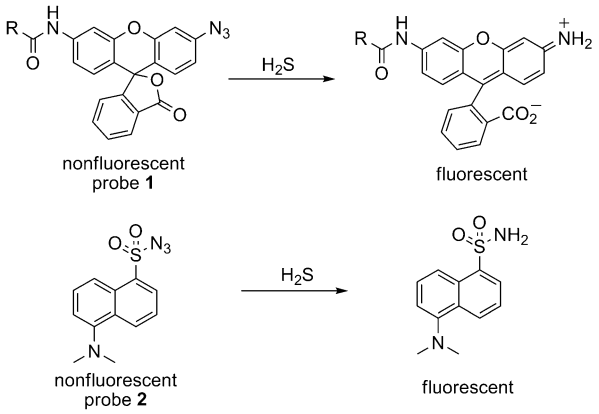
Although hydrogen sulfide ($H_2S$) is known as a toxic gas with the unpleasant smell of rotten eggs, this cannot change the fact that it exists in the human body and other biological systems. It is reported that the typical concentration of $H_2S$ in blood is in the range of 10–100 μM.[1] Clearly its presence must be connected with biological functions, for example, it has been recognized as the third gaseous signaling molecule besides nitric oxide (NO) and carbon monoxide (CO).[2] The significance of endogenously produced $H_2S$ has been validated in a number of physiological and pathological processes, such as the regulation of cell growth,[3] cardiovascular protection,[4] the stimulation of angiogenesis,[5] and antioxidative effects.[6] On the other hand, its deregulation has been correlated with the symptoms of Alzheimer's disease,[7] Down's syndrome,[8] diabetes,[9] and liver cirrhosis.[10] As a result of the huge potential benefits of understanding the biological functions of $H_2S$, recent research on $H_2S$ is gaining considerable attention.[11] Accordingly, sensitive and selective detection techniques that enable the distribution and function of $H_2S$ in complicated biological systems to be probed are highly valuable.

Current methods that are available for the detection of $H_2S$ include colorimetric,[12] electrochemical,[13] and gas chromatography assays.[14] However, these techniques do not allow the temporal and spatial monitoring of reactive and transient $H_2S$. Furthermore, tedious and complicated preparation of the samples is required. Although fluorescent imaging methods are highly desirable and offer high sensitivity as well as real-time imaging, no such probes were available until recently (a previously reported probe is far from meeting current requirements[15]), when the three research groups of Chang,[16] Wang,[17] and Xian[18] disclosed their studies independently, at almost the same time. In this Highlight, these important discoveries are summarized.[19,20]

The designs of the three fluorescent probes are based on altering the fluorescent properties of the probes (for example, the fluorescence intensity) by taking advantage of the reducing or nucleophilic properties of $H_2S$. In the first two approaches,[16,17] the reduction of a fluorophore-tethered azide with $H_2S$ leads to the generation of an amine, and thus induces an increase in fluorescence. In the design from Chang and co-workers, rhodamine analogues are masked by an azido group, and the closed lactone form **1** is not fluorescent (Scheme 1).[16] In the presence of $H_2S$, the azide is selectively reduced to an amine with concomitant ring opening of the lactone to form the highly fluorescent conjugated open form. These rhodamine derivatives have a *tert*-butyloxycarbonyl (Boc) group or a morpholine carbamate for protecting the other amino moiety and are able to detect $H_2S$ in aqueous solution and living cells. Furthermore, imaging of $H_2S$ in vitro at a concentration as low as 5–10 μM was achieved. However, there are also some drawbacks. Firstly, it takes about an hour to reach the highest level of fluorescence enhancement, and this may present problems for the detection of $H_2S$ in real-time because $H_2S$ is metabolized rapidly. Secondly, during




***Scheme 1.*** Probes for $H_2S$ based on azide-masked fluorophores.

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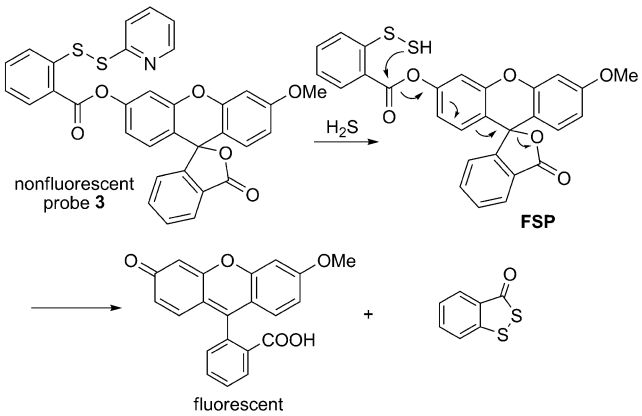
detection over a long time period, other reactive species could participate in reactions with the azide, which could result in low selectivity. For example, the probe is also responsive to superoxide ($O_2^-$). Modifications to the probes may generate a useful imaging system with practical applications.

Almost at the same time, Wang and co-workers reported an interesting probe for detecting $H_2S$ that uses a similar strategy of the reduction of azide **2** to an amine (Scheme 1).[17] In this approach, the azide moiety is directly attached to the sulfonyl group of a dansyl fluorophore. The dansyl azide **2** is not fluorescent. In the detection process, $H_2S$ selectively converts **2** into a highly fluorescent dansyl sulfonamide with high specificity. Notably the probe is not reactive with other commonly found biothiols, such as cysteine and glutathione, and does not undergo displacement reactions with amino groups or other nucleophilic anions, nor does it react with reducing species, even at very high concentrations (1.0 mM, Scheme 2). Furthermore, a significant increase in fluorescence is observed. In a phosphate buffer solution, a 40-fold enhancement in fluorescence occurs with 25 μM $H_2S$. The probe detects $H_2S$ at concentrations as low as 1 μM with a noticeable change in fluorescence intensity (signal/noise ratio of 3:1). Unlike probe **1**, probe **2** responds to $H_2S$ very quickly. In bovine serum at 30 μM $H_2S$, the highest enhancement in fluorescence is observed within seconds. This is attributed to the adjacent electron-withdrawing sulfonyl group, which reduces the electron density of the azido group and, therefore, increases its reactivity towards $H_2S$. Furthermore, a good linear relationship is observed between the enhancement in fluorescence and the concentration of $H_2S$ in buffer solution and in bovine serum. Impressively, the probe enables the concentration of $H_2S$ in mouse blood to be quantified, and the result of $(31.9 \pm 9.4)$ μM is consistent with data that was obtained by using other methods.[20] These features, plus an easy method of preparation, make the probe very attractive for measuring the distribution and contribution of $H_2S$ in biological systems. This probe has huge potential for practical applications.




***Scheme 2.*** Probe for $H_2S$ based on unmasking fluorescein with $H_2S$.

The third probe (**3**, Scheme 2) is described by Xian and co-workers and uses a different reaction mechanism from that of the previous two probes.[18] In this design, $H_2S$ is employed as a nucleophile for the substitution of the thiopyridine moiety to generate a S–SH group. The resulting S–SH undergoes cyclization to produce a fluorescent molecule. As shown in Scheme 2, a fluorescein fluorophore is masked by an ester group with an adjacent disulfide bond, and is not fluorescent. The nucleophilic substitution reaction between this masked fluorescein and $H_2S$ at the disulfide bond leads to the formation of substitution product **FSP**, which spontaneously undergoes cyclization to cleave the masking ester moiety. The fluorescein is released with concurrent recovery of fluorescence. Probe **3** can detect $H_2S$ with a good linear relationship between the intensity of the fluorescence and the concentration of $H_2S$ when the concentration of $H_2S$ is lower than 10 μM. Moreover, it has been applied as a probe for $H_2S$ in complex biological media, such as bovine plasma, and to visualize $H_2S$ in living cells. Unfortunately, no experiments were designed for tracking endogenously produced $H_2S$ with **3**. This may be a result of the limited sensitivity and slow reaction (an hour to reach the maximum fluorescence intensity). Furthermore, the main issue for this system is the side reactions of biothiols (cysteine derivatives) with **3**. A similar nucleophilic substitution can occur to generate a nonfluorescent product. Although the by-product is not fluorescent, it can compete with $H_2S$ to react with **3**. This may result in low sensitivity and require a higher concentration of **3** to achieve desired signal. Moreover, the labile ester linkage may induce a high background signal in biological assays, as it can be cleaved by proteases and esterases to produce fluorescein. Therefore, the sensitivity of **3** is negatively influenced by the complexity of the nucleophilic substitution reaction. The properties of fluorescent probes **1**, **2**, and **3** are summarized in Table 1.

***Table 1:*** Summary of fluorescent probes for the detection of $H_2S$.[a]

| Probe | Detection mechanism, time | Detection medium, limit | Fluorescence enhancement | Detection in living cells | Detection of endogenous $H_2S$ |
|---|---|---|---|---|---|
| **1** | azide reduction, 1 h | HEPES, 5-10 μM | 7–9-fold at 100 μM | yes | no |
| **2** | azide reduction, seconds | bovine serum, 1 μM | 40-fold at 100 μM | N/A | yes |
| **3** | nucleophilic substitution, 1 h | PBS/MeCN, ca. 2.5 μM | 55–77-folds at 50 μM | yes | no |

[a] HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid, N/A = not available, PBS = phosphate-buffered saline.

In conclusion, these three studies make the detection of $H_2S$ by using fluorescence possible. More importantly, these techniques will enable biomedical researchers to investigate the properties and functions of $H_2S$ in biological systems. Furthermore, it is expected that the design strategies described here will trigger the development of new and improved probes to meet specific research needs and for practical applications.

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