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Aldehyde group assisted thiolysis of dinitrophenyl ether: a new promising approach for efficient hydrogen sulfide probes†

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

We report herein a new approach, which combines fast nucleophilic addition of H₂S to an aldehyde group and the subsequent intramolecular thiolysis of dinitrophenyl ether, can be used to develop efficient and effective H₂S probes.

Much attention has been given in the last few years to the development of small molecular probes for hydrogen sulfide (H₂S) since the biological significance of H₂S was recognized recently. Among them, fluorescent detection method is highly attractive due to its simplicity, high sensitivity, low cost and ability of real-time detection. Accordingly, highly selective and sensitive fluorescent probes with rapid response for H₂S under mild conditions become in high demand, as they have huge potential benefits for a better and deeper understanding of the biological functions of H₂S.

Since 2011, a number of fluorescent probes for H₂S have been developed based on several significant chemical properties of H₂S, such as good reducing property, high binding affinity towards copper ions, specific nucleophilic addition to unsaturated double bonds, dual nucleophilicity as well as efficient thiolysis of dinitrophenyl ether. Among them, using the dual nucleophilicity of H₂S is especially attractive for H₂S probes because high selectivity for H₂S can be well guaranteed by this strategy. Although the advantage of this strategy has been applied in several innovative H₂S probes, most of them showed a delayed response time (typically > 20 min) to detect H₂S. Actually, this is a common problem for most reaction-based H₂S probes reported to date, which is a disadvantage for efficient capturing of the transient H₂S. Besides, some other drawbacks of these reported probes can be also found such as either they need a relatively high probe loading due to the probe consumption by other thiols, or need complicated synthetic works, or need to use surfactants to promote the reactions. Therefore, new methods are highly expected to overcome these shortcomings.

We are interested in the thiolysis of dinitrophenyl ether (A in Scheme 1), and they generally suffered from low reaction rates and thus long response times are also needed. Although addition of surfactant such as cetyltrimethylammonium bromide (CTAB) or Tween-20 can enhance the reaction rates significantly, the use of surfactant limits the applications of these probes. Since intramolecular reaction can perform more efficiently than an intermolecular one, we thought that the thiolysis reaction could become much faster if it was switched into an intramolecular fashion. Using the unique dual nucleophilic characters of H₂S, we envisaged that combining the fast nucleophilic addition of H₂S to an aldehyde group and the subsequent spontaneous intramolecular instead of intermolecular thiolysis of dinitrophenyl ether could achieve this purpose (B in Scheme 1). Motivated by this concept, we herein report that this new approach can be very promising to develop efficient H₂S probes.

To investigate the feasibility of our design concept, we chose a known excited state intramolecular proton transfer (ESIPT) dye, compound 3 in Scheme 1 as the fluorophore, because this type dye has been widely used to construct fluorescent probes due to their large Stokes shift and good photostability. In addition, it can be easily prepared, and an aldehyde group can be also easily introduced to the ortho position of the hydroxyl group in this dye to afford an aldehyde containing fluorophore 4. Thus, two new probes (probe 1 and 2) for the purpose of this study can be readily obtained by protection of the hydroxyl group in 3 and 4 with a dinitrophenyl group, respectively. The synthesis of these two probes is illustrated in Scheme S1 (ESI†), and their structures are confirmed by NMR, IR and HR-MS analysis. Detailed synthetic procedures and structure characterizations are given in the
Since 3 and 4 are the expected thiolysis products of probe 1 and 2 with H$_2$S, respectively, we first checked the differences in their optical spectra. As shown in Fig. S1 (ESI†), 3 absorbs light in the UV range ($\lambda_{max}$ 335 nm), however, $\lambda_{max}$ of 4 red-shifted to visible light region ($\lambda_{max}$ 445 nm), which indicates 4 is visible light excitable. Fluorescence spectra showed that both probe 1 and 2 are non-fluorescent, while 3 and 4 show much stronger fluorescence. Besides, 4 shows not only longer emission wavelength, but also higher fluorescence quantum yield than 3 (4: $\lambda_{em}$ 545 nm, $\Phi$ 0.18 vs 3: $\lambda_{em}$ 480 nm, $\Phi$ 0.04), indicating 4 is a better fluorophore (Fig. S1b, ESI†). Anyhow, if the thiolysis reactions of both probes with H$_2$S are fast, then both of them should give rapid and significant optical signal changes.

The sensing ability of probe 1 and 2 for H$_2$S was then investigated in 20 mM PBS buffer (pH 7.4) with 25% CH$_3$CN (v/v) at 25°C. Under this condition, we observed that addition of 10 equiv of NaHS (a standard source for H$_2$S) to the probe 1 and 2 (20 µM of each) solutions resulted in significant differences. As shown in Fig. 1, the probe 1 solution showed very small changes upon addition of NaHS even the reaction time was prolonged to 18 h. In contrast, the probe 2 solution showed fast and significant optical changes within a few minutes upon addition of NaHS, and meanwhile, the colorless solution of probe 2 changed into yellow, and gave off strong green fluorescence as compound 4. Kinetic studies showed that the reaction (20 µM probe 2 + 100 µM NaHS) completed at about 2 min (Fig. S2, ESI†). The observed pseudo-first-order rate constant ($k_{obs}$) at 25°C was determined to be about 2.077 min$^{-1}$ ($t_{1/2}$ ≈ 0.334 min) and the emission intensity was found to increase about 120-fold at 545 nm. Since the reaction between probe 1 and NaHS cannot complete even after 18 hours under the same conditions (Fig. S2, ESI†), probe 2 responses at least more than hundreds of times faster than probe 1 for H$_2$S, indicating our design concept works very well. In fact, probe 2 is able to detect H$_2$S more rapidly than most of the reported reaction-based probes for H$_2$S.$^{1,2,3,5,6}$ In addition, it was also found that probe 2 can work with less amount of CH$_3$CN and can work over a wide range pH with the optimal pH around 7.0 (Fig. S3, ESI†). All these results indicate that this new combined approach is effective and attractive.

To confirm the thiolysis of dinitrophenyl ether and releasing of 4, the reaction product of probe 2 with NaHS was isolated and subjected to thin layer chromatography (TLC) and $^1$H NMR analysis, and the results proved that the product is 4 (Fig. S4-5, ESI†). Considering the aldehyde group in probe 2 brings larger steric hindrance to the dinitrophenyl ether moiety for a direct thiolysis by H$_2$S and the low reactivity of probe 1 for H$_2$S, the sensing process of probe 2 for HS$^-$ was most likely happened as route B shown in Scheme 1.

The above results show that probe 2 can be used as a rapid and sensitive dual colorimetric and fluorescent turn-on sensor for HS$^-$ in aqueous solution. To shed further light on the sensitivity of probe 2 for H$_2$S, the changes in its fluorescence were investigated by addition of various concentrations of NaHS. As shown in Fig. S6 (ESI†), probe 2 is reasonably stable under the test conditions, and upon addition of 0.6-10 equiv HS$^-$, the fluorescence intensity of the probe 2 solutions all showed significant enhancement within 5 min. When the reaction was monitored after 5 min of HS$^-$ addition, a saturation of fluorescence can be observed after HS$^-$ was added more than 2 equiv (Fig. S7, ESI†). In addition, we also observed a good linearity between the fluorescence intensity at 545 nm and the concentration of HS$^-$ in the range of 0 to 12 µM (Fig. 2), thus, the detection limit (S/N = 3) of probe 2 for HS$^-$ is calculated to be about 48 nM under the test conditions. This indicates probe 2 has high sensitivity for H$_2$S.

We then investigated the selectivity of probe 2 for HS$^-$ over various competitors such as F$_-$, Cl$_-$, Br$_-$, I$_-$, NO$_2$-, NO$_3$-, AcO$_-$, SCN$^-$, CO$_2$$_2$, HCO$_2$-, H$_2$PO$_4$-, CN$^-$, SO$_4^{2-}$, HSO$_3$-, SO$_3^{2-}$, HSO$_4$-, SO$_2$O$_3^{2-}$, SO$_2$O$_4^{2-}$, cysteine (Cys), homocysteine (Hcy), glutathione (GSH), N-acetyl-cysteine (NAC), C$_6$H$_5$NH$_2$, C$_6$H$_5$CH$_2$NH$_2$, HCN, CH$_3$CH$_2$NH$_2$ and HOCH$_2$CH$_2$NH$_2$, and the results showed that probe 2 is highly selective for HS$^-$ as shown in Fig. 3 and Fig. S8-9 (ESI†), only addition of HS$^-$ resulted in significant optical changes to the probe 2 solution. In contrast, addition of other analytes showed almost no effect. Notably, detection of HS$^-$ using probe 2 in the presence of these analytes are still effective (Fig. S10, ESI†), even biothiols Cys, Hcy, GSH and NAC are present in a millimolar range (Fig. S11, ESI†). Despite the fact that aryl-aldehydes have been reported to react with biothiols such as Cys to form stable thiazolidines, our
experiments showed that compound 4, the product of probe 2 with HS− is not fluorescent responsive to Cys even they were incubated more than 10 hours (Fig. S12, ESI†). Therefore, all these results indicate that probe 2 is highly selective for HS−.

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Notes and references


