A Specific Nucleophilic Ring-Opening Reaction of Aziridines as a Unique Platform for the Construction of Hydrogen Polysulfides Sensors

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Supporting Information

ABSTRACT: A hydrogen polysulfide mediated aziridine ring-opening reaction was discovered. Based on this reaction, a novel H2Sₙ-specific chemosensor (AP) was developed. AP showed high sensitivity and selectivity for H2Sₙ. Notably, the fluorescent turn-on product (1) exhibited excellent two-photon photophysical properties, a large Stokes shift, and high solid state luminescence efficiency.

Reactive sulfur species (RSS), including biothiols, hydrogen sulfide (H₂S), sulfane sulfur, and S-modified cysteine adducts (i.e., S-nitrosothiols, sulfenic acids, etc.), play important roles in redox biology.¹⁻⁴ Among these species, hydrogen polysulfides (H₂Sₙ, n > 1) have recently received particular attention as they are believed to be involved in H₂S-mediated signaling transduction.⁵⁻¹⁰ Although H₂S has been extensively studied as a new signaling molecule in the past decade, the fundamental chemistry and physiological function of H₂Sₙ are still poorly understood. H₂Sₙ belong to the sulfane sulfur family and have very unique chemistry. H₂Sₙ can be derived from endogenous H₂S by the action of reactive oxygen species,¹¹⁻¹³ as redox partners of H₂S. In this regard, H₂Sₙ very likely coexist with H₂S in vivo and they work together to regulate sulfur redox balance. Some biological activities that were originally ascribed to H₂S may actually be mediated by H₂Sₙ. One such case is S-sulfhydration (i.e., conversion of protein cysteine residues (−SH) to persulfides (−S−SH)).¹⁴⁻²⁰ This reaction was originally ascribed to H₂S. However, H₂Sₙ are recently found to be more effective than H₂S in S-sulfhydration.⁵⁻¹⁰

The field of H₂Sₙ is now rapidly growing, and more exciting biological activity exhibited by H₂Sₙ are to be discovered. In order to better understand the roles of H₂Sₙ, it is critical to develop efficient methods that can distinguish H₂Sₙ from other reactive sulfur species, especially H₂S and biothiols. This is still an underdeveloped field. So far the most commonly used method for H₂Sₙ detection is to measure UV absorption at 290–300 and 370 nm. This low-sensitivity method is unsuitable for biological samples. Fluorescence-based methods could be ideal due to their rapid, sensitive fluorescent responses and spatiotemporal resolution capability.²²⁻²³ Our laboratory recently discovered a H₂Sₙ-mediated aromatic substitution–cyclization and reported the first H₂Sₙ-specific fluorescent sensors based on this reaction.²⁴ The sensors utilize 2-fluoro-5-nitro-benzoic ester to trap H₂Sₙ and release the fluorophores. Although the sensors proved to be highly selective for H₂Sₙ, the competing reaction of the 2-fluoro-5-nitro-benzoic ester template with biothiols could cause the consumption of the sensors and higher sensor loading may be required. To solve this problem, further improvements of the sensor template or the discovery of novel H₂Sₙ specific reaction templates would be needed. Herein we report a unique reaction between aziridines and H₂Sₙ. Based on this reaction, a novel H₂Sₙ-specific sensor (AP) was prepared and evaluated. The physical properties of the actual fluorescent species generated from the reaction of H₂Sₙ were also studied.

In order to develop reaction-based fluorescent sensors for H₂Sₙ detection, the key is to identify specific reactions that only react with H₂Sₙ but not react with biothiols such as glutathione (GSH) and cysteine (Cys) (which are ubiquitous in biological systems and concentrations can reach mM levels). The

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presence of H2S (at μM levels) should also be taken into consideration. Due to α-effects, H2S are expected to be weak acids. The estimated pKa values of H2S are in the range of 3 to 5.25,26 For comparison, the pKa values of H2 and biothiols are in the range of 7 to 9.2 (H2S, 7.0; Cys, 8.30; GSH, 9.2). Under physiological pH, H2S should be stronger and more reactive nucleophiles than biothiols and H2S. As such, our focus was on possible electrophiles which are reactive enough for H2S but not reactive toward H2S and thiols.

Aziridines are popular electrophilic synthons for making amine-containing molecules. Ring-opening reactions of aziridines by many nucleophiles, including thiol-derivatives, have been reported.27–30 In most cases the reactions were for synthetic purposes. High concentrations of reactants, organic solvents/bases, long reaction times, and elevated temperatures are often employed. It should be noted that although those studies revealed good reactivity of aziridines toward certain nucleophiles such as thiols, whether or not such reactions could be used for the detection of thiols or related biological molecules are still unclear. The low concentrations of analytes in biological systems and the mild, neutral, and aqueous environments may make the reactions slow and nonproductive so the reaction signals cannot be visualized. So far the use of aziridine-based sensors for the detection and distinction of reactive sulfur species has not been well studied. We wondered if the strong nucleophilicity of H2S under physiological conditions could be recognized by activated aziridine-based chemosensors. A N-sulfonylaziridine chemosensor was selected for this study.

In the design of the proposed chemosensor, we expected the aziridine moiety and strongly fluorescent dansyl group to be the recognition unit and signaling unit, respectively. The fluorescence of dansyl group should be effectively quenched or weakened via the twisted intramolecular charge transfer (TICT) effect. If the aziridine ring of the sensor could be opened upon reacting with H2S, the resultant dansylamide derivative should possess strong fluorescence. With this idea in mind, an off-on fluorescent chemosensor, AP, was prepared from a simple coupling between dansyl chloride and 2-methylaziridine (Scheme 1).

### Scheme 1. Preparation of AP

![Scheme 1](image)

With the sensor in hand, we first tested the time-dependent fluorescence changes of AP (10 μM) in the absence and presence of H2S (25 μM). Freshly prepared Na2S2 was used in buffer solutions as the equivalent of H2S. As shown in Figure 1A, AP showed very weak fluorescence with a low quantum yield (Φ = 0.01) in PBS buffer (pH 7.4), which was due to the TICT effect. The sensor appeared to be quite stable, as no fluorescence change was noted in 45 min. Upon the treatment of Na2S2, the fluorescence intensity at 530 nm increased dramatically, presumably due to a ring-opening reaction and the formation of the dansylamide derivative. This was also accompanied by a distinct fluorescence color change from weak yellow to bright green (see the inset of Figure 1A), which might be useful for the simple detection of H2S by the naked eye. The maximum intensity could reach a plateau in 15 min, suggesting this was a fast detection. To ensure reproducibility, a reaction time of 30 min was used in all the experiments in this study. It should be noted that 50 μM CTAB was used in our experiments. Without CTAB, the reaction between AP and H2S was found to be slow and nonproductive. This might be due to the poor stability of H2S in water. The use of CTAB significantly increased reaction rates. The effects of CTAB could be attributed to the fact that (1) CTAB can increase the solubility of the sensor in aqueous buffers and (2) CTAB is a cationic surfactant, which may absorb a polysulfide anion (HS−) and facilitate the reaction. We next studied the fluorescence responses of AP to Na2S2 at varied concentrations. Upon gradual introduction of Na2S2 (1 to 40 μM), increases in fluorescence emission at 530 nm were observed. This spectroscopic property displayed a large Stokes shift (180 nm), which should prevent serious self-quenching and fluorescence detection error due to excitation backscattering effects. We also noted that the fluorescence intensity increased linearly with the concentrations of Na2S2 in the range of 0–20 μM. The detection limit31,32 was calculated to be 0.3 μM, suggesting the sensor was highly sensitive to H2S. In addition, we studied the pH effects on this reaction and found that AP was a stable sensor in aqueous buffers and worked effectively in a pH range from 6 to 10 (Figure S1 in the Supporting Information).

Having proven the sensitivity of AP toward H2S, we then turned our attention to examine the specificity of AP. In this study AP (10 μM) was treated with a series of biologically relevant sulfur species including GSH, Cys, Hcy, GSSG, H2S, SO3−, SO2−, CH3SSCH3, and S0. The concentrations of these species were selected based on their biological relevance. As shown in Figure 2, none of these molecules gave a significant fluorescence response (columns 1–9). We also tested the responses of AP to common reactive oxygen species and some representative amino acids, including hydrogen peroxide (H2O2), hypochlorite (ClO−), superoxide (O2−), the hydroxyl radical (·OH), singlet oxygen (O2·), alanine, proline, serine, lysine, tryptophan, and histidine. Again these species did not exhibit any significant fluorescence response (columns 10–20). H2S are highly reactive species and may react with thiols to form persulfides. We then wondered if AP could effectively

![Figure 1](image)
identify H$_2$S$_n$ in the presence of thiols. To this end, AP was applied in the mixtures of Na$_2$S$_2$ and thiols (columns 21–23). We still observed satisfactory fluorescence increases, albeit the values ($\Delta F$) were lower as compared to that of Na$_2$S$_2$ only. This could be attributed to the loss of H$_2$S$_n$ in the reactions with thiols.\(^6,7,13,33\) Moreover, we tested the detection of in situ synthesis of AP and H$_2$S$_n$ formation by AP. Nagy et al. reported that hypochlorite (ClO$^-$) could rapidly react with H$_2$S to form H$_2$S$_2$.\(^11\) When Na$_2$S (100 $\mu$M) and ClO$^-$ (25 $\mu$M) coexisted, AP gave a very strong fluorescence enhancement (column 25) and the value was even higher than that of Na$_2$S$_2$. Taken together, these results demonstrated the specificity and sensitivity of AP for H$_2$S$_n$.

To understand the fluorescence turn-on mechanism, the reaction between AP and Na$_2$S$_2$ was studied at the semi-synthetic scale (60 mM AP; 68 mM Na$_2$S$_2$) (Scheme 2). The reaction was found to be fast which completed within 30 min at room temperature. The major isolated product was disulfide 1 (60% yield). This result indicated that H$_2$S$_n$ could effectively open the azidine ring of AP to form an intermediate 11. It was possible that 11 reacted with another molecule of AP to form the final product 1. Another possibility was that 11 decomposed to form polysulfide 12, which was eventually converted to a stable disulfide product.\(^7\) Given the good yield obtained in this reaction, we expected it could be used for the synthesis of disulfide derivatives.

The isolation of the disulfide product 1 allowed us to carefully study its fluorescence properties as a new fluorophore. As shown in Figure 3, this molecule displayed a strong green fluorescence with a moderate quantum yield ($\Phi = 0.25$) in PBS buffer (pH 7.4). In addition, compound 1 exhibited interesting two-photon photophysical properties. The shape of the two-photon emission spectrum ($\lambda_{ex} = 740$ nm) closely resembles that obtained by single-photon excitation ($\lambda_{ex} = 350$ nm) (Figure 3A and Figure S2 in the Supporting Information). This characteristic suggested that AP might be a useful two-photon fluorescent sensor. Of particular interest is that compound 1 is a highly emissive fluorophore in solid states, such as in powder form and spin-coated thin films, and even in poly(methyl methacrylate) (PMMA) films. Both powder form and a spin-coated thin film of 1 were found to have bright green fluorescence ($\lambda_{em} = 502$ nm) under UV light ($\lambda_{ex} = 365$ nm). A PMMA film dispersing 0.8% compound 1 also emitted a strong blue-green fluorescence ($\lambda_{em} = 466$ nm), bearing the excellent efficiency of the solid-state emission ($\Phi = 1.00$, calculated by using an integrating sphere$^{34,35}$). The development of organic molecules bearing high solid state luminescent efficiency with a high absolute quantum yield remains a difficult task in the field of optoelectronic devices.\(^36\) Currently available molecules with such properties are still quite scarce. Compared to most known organic solid-state luminescence molecules, the unique properties of fluorophore 1, such as good solubility, easy and low-cost synthesis, and high absolute quantum yield, may endow it as a potential candidate for organic emitters and for application to solid-state lighting devices.

To further evaluate the application of AP in biological samples, the detection of H$_2$S$_n$ in diluted deproteinized bovine plasma\(^37\) was performed (Figure S3 in the Supporting Information). Upon gradual introduction of Na$_2$S$_2$, steady fluorescence enhancements appearing at 530 nm were observed. The fluorescence response signals were lower than those obtained in PBS buffers. This may be due to the consumption of H$_2$S$_2$ by biothiols. Nevertheless a good linear correlation between the H$_2$S$_2$ concentration and the fluorescence intensity at 530 nm was obtained.

Figure 2. Fluorescence intensity increases ($\Delta F$) of AP (10 $\mu$M) in the presence of various RSS, ROS, amino acids. (1) 10 mM GSH; (2) 500 $\mu$M Cys; (3) 100 $\mu$M Hcy; (4) 100 $\mu$M GSSG; (5) 100 $\mu$M Na$_2$S$_2$; (6) 100 $\mu$M Na$_2$S; (7) 100 $\mu$M Na$_2$SO$_3$; (8) 100 $\mu$M CH$_3$SSCH$_3$; (9) 100 $\mu$M $\text{S}_2$; (10) 250 $\mu$M H$_2$O$_2$; (11) 25 $\mu$M ClO$^-$; (12) 25 $\mu$M O$_3$; (13) 25 $\mu$M OH$^-$; (14) 25 $\mu$M I$_2$O$_5$; (15) 100 $\mu$M alanine; (16) 100 $\mu$M proline; (17) 100 $\mu$M serine; (18) 100 $\mu$M lysine; (19) 100 $\mu$M tryptophan; (20) 100 $\mu$M histidine; (21) 100 $\mu$M GSH + 50 $\mu$M Na$_2$S$_2$; (22) 100 $\mu$M Cys + 50 $\mu$M Na$_2$S$_2$; (23) 100 $\mu$M Hcy + 50 $\mu$M Na$_2$S$_2$; (24) 25 $\mu$M Na$_2$S; (25) 25 $\mu$M ClO$^-$ + 100 $\mu$M Na$_2$S.

Figure 3. (A) Two-photon fluorescence emission spectra of AP (red line) and 1 (black line) ($\lambda_{em} = 740$ nm). (B) Fluorescence emission spectra of 1 in different forms and corresponding fluorescence photos under UV light ($\lambda_{ex} = 365$ nm): (a) PBS buffer (pH 7.4); (b) solid powder; (c) spin-coated thin films; (d) PMMA solid films (film thickness is 50 $\mu$m) doped with 0.8% compound 1.
In summary, we have reported a unique ring-opening reaction of N-sulfonylaziridine by Na2S2 under mild conditions. This reaction was used to develop a specific fluorescent sensor AP for the detection of H2S. The sensor was found to be selective and sensitive for H2S while other reactive sulfur/oxygen species and amino acids could not turn on the fluorescence. Moreover, the fluorophore, i.e. compound 1, exhibited excellent two-photon photophysical properties and a large Stokes shift. Given its high solid state luminescent efficiency, this molecule may be a potential candidate for organic emitters and for application to solid-state lighting devices.

**ASSOCIATED CONTENT**

* Supporting Information

Detailed synthetic procedures, characteristic data, and experimental procedures. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orlett.5b01194.

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**Notes**

The authors declare no competing financial interest.

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