Published in final edited form as:

Free Radic Biol Med. 2015 December ; 89: 662–667. doi:10.1016/j.freeradbiomed.2015.08.017.

Reactions of Isolated Persulfides Provide Insights into the Interplay between H_2S and Persulfide Reactivity

T. Spencer Bailey and Michael D. Pluth*

Department of Chemistry and Biochemistry, Institute of Molecular Biology, Materials Science Institute. University of Oregon, Eugene, OR 97403, USA

Abstract

Hydrogen sulfide is ubiquitous in biological systems and exerts function over a wide range of important physiological processes. Complementing free H_2S , the reductant-labile sulfur pool plays significant roles in the translocation and action of sulfide, however the chemistry of reductant-labile sources of sulfide have not been studied systematically. Using a combination of NMR and UV-Vis spectroscopy, we investigated the spectroscopic properties and reactivity of three isolated organic persulfides and report a simple model for persulfide reactivity, including their roles as nucleophiles, electrophiles, and sulfide donors.

graphical abstract

Keywords

Hydrogen sulfide; persulfides; polysulfides; sulfane sulfur; redox chemistry

1. Introduction

Hydrogen sulfide (H_2S) continues to generate significant interest as an important biomolecule due to its role as a ubiquitous signaling molecule in diverse biological systems and processes.[1, 2] Produced by both enzymatic[3] and non-enzymatic processes, H_2S

*corresponding author. pluth@uoregon.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Supporting Information

Supplementary data associated with this article can be found in the online version at (insert address here).

plays important roles in maintaining immune, cardiovascular, neuronal, as well as other functions.[1, 4] Although sulfide signaling mechanisms remain an active area of investigation, they also face significant challenges due to the inherent complexity of the chemical processes occurring during the course of such mechanisms. Cellular concentrations of free H_2S are now understood to be in the low to mid-nanomolar range, [5] which is significantly lower than the initial reports of physiologically-unreasonable micromolar levels of free sulfide.[6, 7] This significant revision of endogenous sulfide levels is due in part to better tools for measuring and differentiating sulfide pools, and also reflects a broadened appreciation of the importance of reductant-labile forms of sulfide.[8] Contrasting acid-labile sulfide pools, which often consist of metal sulfides such as iron sulfur clusters,[9-11] the reductant-labile sulfane-sulfur pools are comprised primarily of oxidized thiols such as hydrodisulfides/persulfides ($RSSH$), hydropolysulfides (RSS_nSH), and both organic and inorganic polysulfides ($RS(S)_nSR$).[2, 12-15]

The reductant-labile sulfane sulfur pool is hypothesized to play significant roles in the observed biological action associated with H_2S and also provides access to redox-neutral pathways for transsulfuration and transpersulfidation processes.[16, 17] In addition to providing a significant source of redox-labile sulfide, proteins bearing persulfide modified cysteine residues show altered activity, such as increased nucleophilicity, by comparison to the parent thiols.[18-23] Persulfides may also play important roles in the observed antioxidant properties of H_2S , based on the higher stability of the persulfide radical ($RSS\bullet$) compared with the thiyl radical ($RS\bullet$).[24]

Despite the widespread importance of persulfides in biological processes, understanding the different reactivity of persulfides, thiols, and sulfide remains a significant challenge, in part due to the inherent complexity of the sulfane sulfur pool and in part due the difficulty of disentangling the innate chemistry of its constituents, because the chemistries of persulfides, thiols, and sulfide are largely intertwined. Further complicating our understanding of persulfide chemistry, many of the previous studies on persulfides have relied on persulfides generated in situ rather than purified compounds.[25-28] Although isolated persulfides have appeared sporadically in the literature and have been the focus of previous studies,[29] little work has been done to systematically characterize persulfide reactivity under controlled and comparable conditions. The difficulty in differentiating persulfide reactivity from other sulfhydryl-containing biomolecules has been reflected by the significant challenges in designing selective methods for labeling persulfides.[30-32] A more complete understanding of the inherent chemical properties and reactivity of persulfides would allow for the development of more reliable persulfide detection methodologies and would also provide valuable insights into the roles of persulfides in biological signaling and regulation. To address this need, we have synthesized three organic persulfides (Figure 1) and characterized their physical properties and reactivity with a variety of reactive small molecules under controlled conditions.

2. Materials and Methods

2.1. Materials

Diacetylsulfide (2), tritylacetyldisulfide (4c), and tritylpersulfide (TrtSSH) were prepared as described previously.[33] Both benzylpersulfide (BnSSH)[34] and adamantylpersulfide (AdSSH)[35] have been reported previously, but we report modified synthetic procedures and complete characterization data below. Thioacetic acid, acetyl chloride, sulfonyl chloride and the required thiols were purchased from commercial suppliers and used as received. Triphenylphosphine, $[\text{NBu}_4^+][\text{CN}^-]$, $[\text{NBu}_4^+][\text{Cl}^-]$, $[\text{NBu}_4^+][\text{I}^-]$, and $[\text{NBu}_4^+][\text{OAc}^-]$ were handled in an inert atmosphere glove box and used as received. $[\text{NBu}_4^+][\text{SH}^+]$ was prepared by reaction of NaSH with $[\text{NBu}_4^+][\text{Cl}^-]$ in MeCN in the absence of both water and air. Concentration of the solution under vacuum, followed by trituration of the resulting solid with Et_2O produces $[\text{NBu}_4^+][\text{HS}^-]$ as a white solid. Dithiopropane was degassed using three freeze-pump-thaw cycles and stored under nitrogen. TEA, was distilled under vacuum prior to use and stored under nitrogen. Trityl thiolate was generated by deprotonation of trityl thiol with sodium hydride. $\text{NO}_{(\text{g})}$ was prepared by the slow addition of H_2SO_4 to a concentrated aqueous solution of NaNO_2 . The evolved gas was purified by passing through a column of NaOH pellets and low-boiling impurities were condensed by passage through a glass wool filled coil at -78°C , and collected in a storage bulb. $\text{H}_2\text{S}_{(\text{g})}$ was purchased from Sigma Aldrich and transferred through a custom-built stainless steel transfer line into a glass storage bulb prior to use. Note: Hydrogen sulfide and its salts are highly toxic and should be handled carefully to avoid exposure. Deuterated solvents were purchased from Cambridge Isotope Laboratories, degassed using three freeze-pump-thaw cycles and stored under nitrogen over $4\text{\AA}$ molecular sieves. Diethyl ether and toluene were degassed by sparging with argon followed by passage through a Pure Process Technologies solvent purification system. All air-free manipulations were performed using standard Schlenk techniques or by use of an Innovative Technology glove box.

2.2 Spectroscopic methods

NMR spectra were acquired on a Bruker Avance-III-HD 600 spectrometer with a Prodigy multinuclear broadband CryoProbe or a Varian INVOA-500 spectrometer at 25.0°C . Chemical shifts are reported in parts per million (δ) and are referenced to residual protic solvent resonances. The following abbreviations are used in describing NMR couplings: (s) singlet, (d) doublet, (b) broad, and (m) multiplet. Persulfide reactivity was assessed by NMR using anhydrous and anaerobic CD_2Cl_2 at room temperature with 18 mM persulfide and reactant (Table 2). UV-vis spectroscopic measurements were performed on a Cary 60 spectrophotometer equipped with a Quantum Northwest cuvette temperature controller under anaerobic conditions in 1.0 cm path length septum-sealed cuvettes obtained from Starna Scientific. IR spectra were measured in the solid phase on a Thermo Scientific Nicolet 6700 RT-IR using an ATR attachment. High resolution mass spectrometry (HRMS) measurements were performed by the Biomolecular Mass Spectrometry Core of the Environmental Health Sciences Core Center at Oregon State University.

2.3 Synthesis of acetyl sulfenylchloride

Acetyl sulfenylchloride (3) was prepared using a procedure modified from the literature [33]. In rigorously dried glassware using anhydrous reagents, sulfuryl chloride (3.73 mL, 46.2 mmol) was added dropwise to diacetylsulfide (5.3 g, 45 mmol) at -30°C in a dry ice/methanol bath under nitrogen. The reaction mixture was warmed from -30°C to -5°C over the course of 1 h, during which time the color of the solution changed from pale yellow to orange. The generated SO_2 was removed at -15°C under vacuum at 20 torr. Vacuum distillation at 100 mtorr of the remaining mixture at 0°C into a receiving flask at -40°C produced 3.10 g (28.0 mmol, 62.3%) of a bright yellow liquid, with spectroscopic properties matching previous reports. This compound was used immediately.

2.4 Synthesis of Acetyldisulfides

General Procedure: The desired thiol (1.82 mmol) was dissolved in 1.0 mL of dry Et_2O and was added dropwise at room temperature to acetylsulfenyl chloride (2, 221 mg, 2.00 mmol) dissolved in 1.0 mL of dry Et_2O under nitrogen. For best results, use freshly distilled 2. The reaction mixture was allowed to stir for 1.5 h. The remaining volatiles were removed under vacuum to give the corresponding acetyl disulfide as a solid. These compounds are all generally stable on the bench top and appear to be stable indefinitely in the freezer.

Benzylacetyldisulfide (4a). The general procedure described above was modified in the following ways: the volatiles were removed at 0°C instead of at rt. Benzylacetyldisulfide was isolated as a pale white solid in 86.5% yield (300 mg). ^1H NMR (600 MHz, CDCl_3) δ : 7.31 (m, 5H), 3.93 (s, 2H), 2.34 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, CDCl_3) δ : 195.2, 136.2, 129.6, 128.7, 127.9, 43.0, 28.8.

Adamantylacetyldisulfide (4b). The general procedure yields 287 mg (57.5%) of a loose white solid. ^1H NMR (600 MHz, CDCl_3) δ : 2.47 (s, 3H), 2.07 (bs, 3H), 1.83 (d, 6H, $J = 2.4$ Hz), 1.66 (q, 6H, $J = 12$ Hz). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, CDCl_3) δ : 195.9, 50.5, 42.2, 36.0, 29.9, 28.9.

2.5 Synthesis of BnSSH and AdSSH

General Procedure: Methanolic HCl (5 N) was prepared by adding acetyl chloride to dry degassed methanol in an ice bath. The acetyldisulfide (100 mg.) was dissolved in 1.0 mL of methanol and cooled in an ice bath under nitrogen. Methanolic HCl (5 N, 342 μL) was added to the solution dropwise, and the resultant reaction mixture was allowed to stir overnight. It is important that no metals be in the reaction vessel at this time. The disulfide does not immediately dissolve fully, and takes up to 3 hr to fully dissolve. After stirring for 24 h, the solvents were removed under vacuum to yield the desired persulfide.

Benzylpersulfide (BnSSH). The general procedure yields 74.0 mg (94%) of a viscous liquid. This compound has a half-life of approximately 2 weeks when stored at -20°C in the absence of both water and oxygen. ^1H NMR (600 MHz, CD_2Cl_2) δ : 7.32 (m, 5H), 3.91 (s, 2H), 2.96 (bs, 1H, SH). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, CD_2Cl_2) δ : 137.5, 129.8, 129.0, 128.0, 43.4. IR (cm^{-1}): 3061, 3028, 2914, 2505, 1493, 1452, 1070, 763, 697, 665. HRMS-ESI (m/z): $[\text{M}-\text{H}]$ calcd for $[\text{C}_7\text{H}_8\text{S}_2]$ 156.0068; found 156.0060.

Adamantylpersulfide (AdSSH). The general procedure yields 74.4 mg (90%) of a viscous oil that quickly solidifies on cooling. Adamantyl persulfide appears stable indefinitely when stored at -20°C , and solution samples do not significantly degrade over the course of a few days. ^1H NMR (600 MHz, CD_2Cl_2) δ : 2.70 (s, 1H, SH), 2.13 (bs, 3H), 1.88 (d, 6H, $J = 2.5$ Hz), 1.73 (q, 6H, $J = 12$ Hz). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, CD_2Cl_2) δ : 46.7, 41.5, 36.0, 30.0. IR (cm^{-1}): 2904, 2849, 2674, 2655, 2491, 1439, 1341, 1311, 1297, 1254, 1102, 1041, 977, 867, 686. HRMS-ESI (m/z): $[\text{M}-\text{H}]$ calcd for $[\text{C}_{10}\text{H}_{16}\text{S}_2]$ 200.0694; found 200.0695.

3. Results and Discussion

We recently reported the isolation, structural characterization, and preliminary reactivity studies of trityl persulfide (TrtSSH).[33] We note that these investigations, as well as those presented herein, were performed in organic solution under anaerobic conditions to enable isolation of specific protonation states of both the persulfide and other reactants as well as to prevent unwanted oxidation chemistry. By contrast to proposed persulfide reactivity with thiols in water,[36] we did not observe H_2S release upon treatment of TrtSSH with thiols. We surmised that this difference could be due to steric constraints of the trityl group, differences in proton transfer in organic versus aqueous solution, or changes in persulfide electronics due to the stability of the trityl cation. For example, the trityl group is partially electron donating due to the high stability of the trityl cation, thus reducing the electrophilicity of the α -sulfur of the persulfide by polarizing C-S bond, which should reduce its reactivity toward thiol nucleophiles. To further investigate these reactivity differences, we have prepared and isolated two additional organic persulfides, benzyl persulfide (BnSSH) and adamantyl persulfide (AdSSH), both of which are known in the literature and have significantly different physicochemical properties than TrtSSH.

AdSSH is similar to TrtSSH in that both are tertiary persulfides. Unlike TrtSSH, however, AdSSH has an entirely aliphatic backbone that is conformationally locked, which significantly reduces the electron donating ability by contrast to TrtSSH. Additionally, AdSSH is significantly less sterically hindered than TrtSSH. To compare the reactivity differences between tertiary and primary persulfides, we also prepared benzyl persulfide (BnSSH), which provides a less sterically hindered persulfide than AdSSH or TrtSSH and also provides an accessible electrophilic α -carbon. If the observed reactivity of these three dissimilar persulfide models provide identical reactivity, then the reactivity differences between biological persulfides and the synthetic persulfides are to be likely attributed to solvents and/or proton transfer chemistry. The described persulfides were synthesized using the general method (Scheme 1). The thiol of the desired persulfide is treated with acetylsulfonyl chloride (3) to yield the corresponding acetyldisulfide (4). Acidic methanolysis of the acetyldisulfide yields the desired persulfide.

With isolated persulfides in hand, we measured and compared their spectroscopic properties and compared these parameters to those of the parent thiols (Table 1). The sulfhydryl ^1H NMR resonance on each persulfide was measured in CD_2Cl_2 and was observed to be 3.02 ppm for BnSSH, 2.70 ppm for AdSSH, and 2.75 ppm for TrtSSH. For both BnSSH and AdSSH, the sulfhydryl proton shifts approximately 1.15 ppm downfield by comparison to the parent thiol, whereas for a 0.35 ppm upfield shift is observed for TrtSSH. Adding to the

spectroscopic characterization, we also measured the S-H stretch of each compound using IR spectroscopy. All three of the persulfides show S-H stretches between 2490 and 2508 cm^{-1} , which in each case is about $\sim 60 \text{ cm}^{-1}$ lower in energy than the parent thiol. The combination of IR and ^1H NMR spectroscopic data provide general spectroscopic characteristics of persulfides when compared to their parent thiols, which should facilitate future characterization and identification of small molecule persulfides.

Having characterized the spectroscopic properties of TrtSSH, AdSSH, and BnSSH, we next systematically investigated the chemical reactivity of the isolated persulfides with a variety of reductants, nucleophiles, bases, and gases (Table 2). Treatment of the persulfides, which contain sulfur atoms in the -1 oxidation state, with one equivalent of triphenylphosphine (PPh_3), a nucleophilic reductant, generates the parent thiol and triphenylphosphine sulfide (Ph_3PS). This reaction constitutes an overall 2-electron reduction and produces two sulfur atoms in the -2 oxidation state. Although TrtSSH and AdSSH react to cleanly afford Ph_3PS and the parent thiols as the only product, BnSSH also generates benzyltrisulfide (BnSSSBn , $\delta = 4.07 \text{ ppm}$) and H_2S ($\delta = 0.9 \text{ ppm}$, Figure S7). The ratio of BnSSSBn to BnSH is 3:4 by ^1H NMR spectroscopy and the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of the reaction mixture shows unreacted PPh_3 in solution, suggesting that the trisulfide-generating reaction pathway does not consume PPh_3 . One explanation for this difference in reactivity is that BnSSH may be sufficiently acidic (vide infra) such that deprotonation of the persulfide by PPh_3 becomes competitive with the rate of PPh_3 -mediated reduction chemistry. Another interesting observation is that BnSSSBn is the only observed polysulfide formed during the reaction, and that higher order polysulfides, such as tetra- and pentasulfides, are not observed. This selectivity is consistent with extraction of sulfur from trisulfides by PPh_3 in methylene chloride being slow[37] by comparison to either higher order polysulfides or persulfides.

Exposure of any of the isolated persulfides to other reducing agents, such as $\text{H}_2\text{S}_{(\text{g})}$, $\text{NO}_{(\text{g})}$, or dithiopropane (DTP) failed to produce any measurable reaction over a 48 hr period. The observed persulfide stability in the presence of DTP and $\text{H}_2\text{S}_{(\text{g})}$ suggests that either these sulfhydryl-containing reductants are either not sufficiently reducing to release sulfide, or that proton transfer steps, which are not favorable in non polar solvents but should be facilitated by solvation in water, are important mechanistic components of the reduction process. To probe whether proton inventory was important to the reduction of persulfides by thiols, we treated AdSSH, TrtSSH, and BnSSH with $[\text{Na}^+][\text{TrtS}^-]$. We observed that instead of acting as a reducing agent and producing H_2S , treatment of the persulfides with $[\text{Na}^+][\text{TrtS}^-]$ produced TrtSH ($\delta = 3.1 \text{ ppm}$, Figures S11, S22), indicating that the thiolate was deprotonating the persulfides and generating a persulfide anion.

To further explore the observed persulfide reactivity, we treated each persulfide with a variety of both anionic and neutral bases. For the series CN^- , AcO^- , Cl^- , and I^- , we observed that persulfides react with the anions at a rate increasing with anion basicity, which is consistent with a mechanism dependent on deprotonation. We observed a slower reaction rate for NEt_3 than with the charged bases, which we attribute to solvation effects. Because the experiments were conducted in CD_2Cl_2 , charged species are solvated much more poorly than neutral species, and this property makes NEt_3 a weaker base by comparison to an anion such as acetate. The reaction of HS^- with BnSSH was fast, however no reaction was

observed with either AdSSH or TrtSSH. If HS^- is acting as a base, it is unclear why the reactivity of HS^- with AdSSH and TrtSSH is so much lower than the other tested bases.

Upon treatment with each of the bases, BnSSH reacted the fastest, followed by TrtSSH, and then AdSSH. We theorize that the increase in reaction rate corresponds to a greater generation of the persulfide anion during the reaction, suggesting that BnSSH is most acidic, followed by TrtSSH, and then AdSSH. Additionally the products observed for the reaction of persulfide with base changed depending on the amount of steric hindrance surrounding the persulfide. AdSSH and TrtSSH are both tertiary persulfides, and in each case their reaction with base produced only the parent thiol and elemental sulfur. By contrast, for the primary persulfide BnSSH, we observed a variety of benzyl polysulfide products ($n=2-6$) in addition to the parent thiol (Table 3). When stronger bases were used, we observed H_2S generation from BnSSH during the reaction.

To verify that the persulfide anion was present over the course of the reaction, we monitored the reaction of the persulfides with base by UV-Vis spectroscopy (Figure 2). Previous work in aqueous buffers above pH 8.0 has shown a peak attributed to the cysteine persulfide anion appears between 335 – 350 nm with a molar absorptivity of $\sim 400 \text{ M}^{-1}\text{cm}^{-1}$, although analytical standards for such compounds remain unavailable.[18, 39] Upon treatment of BnSSH with AcO^- , we initially only observed a minimal change in the large shoulder sweeping down from the UV range. After a short incubation period, however, a peak centered near 340 nm appeared, which we attribute to the persulfide anion, which reaches a maximum intensity of 0.065 AU after 30 min. The solution was monitored for a total of 4 hr, after which the intensity of the peak centered near 340 nm returned to background levels, consistent with complete reaction of the persulfide anion. Further supporting the assignment of the 340 nm absorbance band as a persulfide anion, addition of anhydrous HCl in Et_2O to the reaction mixture immediately abolishes the 340 nm absorbance, which is consistent with protonation of the persulfide anion to generate the parent persulfide. We also observed that the UV-vis reactions proceeded at a significantly slower rate than the NMR experiments, which is consistent with our proposed bimolecular persulfide decomposition reaction because the UV-Vis experiments were conducted at a significantly lower concentration.

On the basis of the observed reactivity of the three persulfides investigated here, we propose a general mechanism for the observed base-mediated decomposition (Figure 3). For the reaction between two persulfides under basic conditions (5), if R is sufficiently sterically hindered or is a tertiary center, such as for AdSSH and TrtSSH, only the terminal sulfur atom of the persulfide is accessible for nucleophilic attack by other persulfides. This reaction results in formation of one equivalent of hydropolysulfide anion (6) and one equivalent of thiol. The resultant hydropolysulfide anions (6) can then react with additional persulfides to further elongate the hydropolysulfide chain (7). Once the sulfur chain in the hydropolysulfide anion is of sufficient length, an intramolecular nucleophilic attack results in extrusion of elemental sulfur (such as S_8) and generates the parent thiol. By contrast, when the R-group is not sterically hindered or is primary center, such as for BnSSH, the α -sulfur atom becomes accessible for nucleophilic attack. Attack on the α -sulfur by a persulfide anion or hydropolysulfide anion results in formation of a symmetrical polysulfide (8) and HS^- , thus terminating the chain.

Conclusions

In summary we have prepared three organic persulfides, TrtSSH, AdSSH, and BnSSH, and investigated their reactivity in aprotic organic solvents using NMR and UV-vis spectroscopic to determine how differences in persulfide structure relate to differences in observed reactivity. Although our experiments were performed in organic solution under anaerobic conditions, and thus may not directly represent the exact reactivity of organic persulfides in aerobic aqueous environments, we believe that the reaction profiles of these isolated persulfides contributes positively to unravelling the complex reactivity associated with biological persulfides. For example we observed that persulfide stability inversely correlates with persulfide acidity, with acidic persulfides decomposing more rapidly than less-acidic persulfides. This observation may help explain why although cysteine persulfides in enzyme active sites act as highly-reactive nucleophiles, less-acidic glutathione persulfide (GSSH) is present and stable in micromolar concentrations in the cytoplasm. Furthermore, differences in solvation and hydrogen bonding environments available to GSSH compared to the cysteine persulfides in enzyme pockets may also contribute to these differences in reactivity. In our model studies, we also observed that the steric bulk near the persulfide significantly impacts the reactivity, and this observation may have consequences in determining the relative electrophilicity and nucleophilicity of the sulfur atoms of the persulfide. This observation may also suggest that specific proteins or protein pockets may be sufficiently shielded to prevent the formation of longer-chain polysulfides, thus imparting selectivity on polysulfide chain length and associated changes in reactivity based on which hydropolysulfides can be generated. Additionally, we observed that persulfides can only release H_2S following reduction by another species, including another persulfide, which supports the general hypothesis that persulfides may play important roles for sulfide storage and transport, whereas free $\text{H}_2\text{S}/\text{HS}^-$ is prone to reaction with other reactive biological species. Taken together, these results provide new insights into persulfide reactivity gleaned from simple model compounds investigated under identical conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This material is based upon work supported by the National Science Foundation (NSF) under CHE-1454747. The NMR facilities at the UO are supported by NSF/ARRA (CHE-0923589). The Biomolecular Mass Spectrometry Core of the Environmental Health Sciences Core Center at Oregon State University is supported, in part, by the NIEHS (P30ES000210) and the NIH.

References

- [1]. Wang R. Physiological implications of hydrogen sulfide: A whiff exploration that blossomed. *Phys. Rev.* 2012; 92:791–896.
- [2]. Li Q, Lancaster JR Jr. Chemical foundations of hydrogen sulfide biology. *Nitric Oxide.* 2013; 35:21–34. [PubMed: 23850631]
- [3]. Maclean, K.; Kraus, J. Hydrogen sulfide production and metabolism in mammalian tissues. In: Wang, R., editor. *Signal transduction and the gasotransmitters*. Humana Press; 2004. p. 275-292.

- [4]. Chen C-Q, Xin H, Zhu Y-Z. Hydrogen sulfide: Third gaseous transmitter, but with great pharmacological potential. *Acta Pharmacol. Sin.* 2007; 28:1709–1716.
- [5]. Furne J, Saeed A, Levitt MD. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2008; 295:R1479–R1485. [PubMed: 18799635]
- [6]. Zhao W, Ndisang JF, Wang R. Modulation of endogenous production of H₂S in rat tissues. *Can. J. Physiol. Pharm.* 2003; 81:848–853.
- [7]. Hosoki R, Matsuki N, Kimura H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem. Biophys. Res. Commun.* 1997; 237:527–531. [PubMed: 9299397]
- [8]. Ishigami M, Hiraki K, Umemura K, Ogasawara Y, Ishii K, Kimura H. A source of hydrogen sulfide and a mechanism of its release in the brain. *Antioxid Redox Signal.* 2009; 11:205–214. [PubMed: 18754702]
- [9]. Clausen T, Kaiser JT, Steegborn C, Huber R, Kessler D. Crystal structure of the cystine C-S lyase from *Synechocystis*: Stabilization of cysteine persulfide for FeS cluster biosynthesis. *Proc. Natl. Acad. Sci. USA.* 2000; 97:3856–3861. [PubMed: 10760256]
- [10]. Ubuka T. Assay methods and biological roles of labile sulfur in animal tissues. *J. Chromat. B.* 2002; 781:227–249.
- [11]. Johnson DC, Dean DR, Smith AD, Johnson MK. Structure, function, and formation of biological iron-sulfur clusters. *Annu. Rev. Biochem.* 2005; 74:247–281. [PubMed: 15952888]
- [12]. Shen X, Peter EA, Bir S, Wang R, Kevil CG. Analytical measurement of discrete hydrogen sulfide pools in biological specimens. *Free Radic. Biol. Med.* 2012; 52:2276–2283. [PubMed: 22561703]
- [13]. Mishanina TV, Libiad M, Banerjee R. Biogenesis of reactive sulfur species for signaling by hydrogen sulfide oxidation pathways. *Nat. Chem. Biol.* 2015; 11:457–464. [PubMed: 26083070]
- [14]. Mueller EG. Trafficking in persulfides: Delivering sulfur in biosynthetic pathways. *Nat. Chem. Biol.* 2006; 2:185–194. [PubMed: 16547481]
- [15]. Greiner R, Pálincás Z, Bäsell K, Becher D, Antelmann H, Nagy P, Dick TP. Polysulfides link H₂S to protein thiol oxidation. *Antiox. Redox Signal.* 2013; 19:1749–1765.
- [16]. Wróbel M, Sura P, Srebro Z. Sulfurtransferases and the content of cysteine, glutathione and sulfane sulfur in tissues of the frog *Rana temporaria*. *Comp. Biochem. Physiol., B: Comp. Biochem.* 2000; 125:211–217.
- [17]. Ida T, Sawa T, Ihara H, Tsuchiya Y, Watanabe Y, Kumagai Y, Suematsu M, Motohashi H, Fujii S, Matsunaga T, Yamamoto M, Ono K, Devarie-Baez NO, Xian M, Fukuto JM, Akaike T. Reactive cysteine persulfides and s-polythiolation regulate oxidative stress and redox signaling. *Proc. Natl. Acad. Sci. USA.* 2014; 111:7606–7611. [PubMed: 24733942]
- [18]. Kessler D. Enzymatic activation of sulfur for incorporation into biomolecules in prokaryotes. *FEMS Microbiol. Rev.* 2006; 30:825–840. [PubMed: 17064282]
- [19]. Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK, Barrow RK, Yang G, Wang R, Snyder SH. H₂S signals through protein s-sulphydration. *Sci. Signal.* 2009; 2:ra72. [PubMed: 19903941]
- [20]. Kabil O, Banerjee R. Characterization of patient mutations in human persulfide dioxygenase (eth1) involved in H₂S catabolism. *J. Biol. Chem.* 2012; 287:44561–44567. [PubMed: 23144459]
- [21]. Paul BD, Snyder SH. H₂S signalling through protein sulphydration and beyond. *Nat. Rev. Mol. Cell. Biol.* 2012; 13:499–507. [PubMed: 22781905]
- [22]. Kimura Y, Mikami Y, Osumi K, Tsugane M, Oka J.-i, Kimura H. Polysulfides are possible H₂S-derived signaling molecules in rat brain. *FASEB J.* 2013; 27:2451–2457. [PubMed: 23413359]
- [23]. Vandiver MS, Paul BD, Xu R, Karuppagounder S, Rao F, Snowman AM, Seok Ko H, Il Lee Y, Dawson VL, Dawson TM, Sen N, Snyder SH. Sulphydration mediates neuroprotective actions of parkin. *Nat. Commun.* 2013; 4:1626. [PubMed: 23535647]
- [24]. Everett SA, Schoeneich C, Stewart JH, Asmus KD. Perthiyl radicals, trisulfide radical ions, and sulfate formation: A combined photolysis and radiolysis study on redox processes with organic di- and trisulfides. *J. Phys. Chem.* 1992; 96:306–314.

- [25]. Ono K, Akaike T, Sawa T, Kumagai Y, Wink DA, Tantillo DJ, Hobbs AJ, Nagy P, Xian M, Lin J, Fukuto JM. Redox chemistry and chemical biology of H₂S, hydropersulfides, and derived species: Implications of their possible biological activity and utility. *Free Radic. Biol. Med.* 2014; 77:82–94. [PubMed: 25229186]
- [26]. Francoleon NE, Carrington SJ, Fukuto JM. The reaction of H₂S with oxidized thiols: Generation of persulfides and implications to H₂S biology. *Arch. Biochem. Biophys.* 2011; 516:146–153. [PubMed: 22001739]
- [27]. Zhao Y, Bhushan S, Yang C, Otsuka H, Stein JD, Pacheco A, Peng B, Devarie-Baez NO, Aguilar HC, Lefer DJ, Xian M. Controllable hydrogen sulfide donors and their activity against myocardial ischemia-reperfusion injury. *ACS Chem. Biol.* 2013; 8:1283–1290. [PubMed: 23547844]
- [28]. Artaud I, Galardon E. A persulfide analogue of the nitrosothiol snap: Formation, characterization and reactivity. *ChemBioChem.* 2014; 15:2361–2364. [PubMed: 25205314]
- [29]. Park C-M, Weerasinghe L, Day JJ, Fukuto JM, Xian M. Persulfides: Current knowledge and challenges in chemistry and chemical biology. *Mol. Biosys.* 2015; 11:1775–1785.
- [30]. Pan J, Carroll KS. Persulfide reactivity in the detection of protein s-sulfhydration. *ACS Chem. Biol.* 2013; 8:1110–1116. [PubMed: 23557648]
- [31]. Zhang D, Macinkovic I, Devarie-Baez NO, Pan J, Park C-M, Carroll KS, Filipovic MR, Xian M. Detection of protein S-sulfhydration by a tag-switch technique. *Angew. Chem. Int. Ed.* 2014; 53:575–581.
- [32]. Galardon E, Padovani D. Reactivity of persulfides toward strained bicyclo[6.1.0]nonyne derivatives: Relevance to chemical tagging of proteins. *Bioconj. Chem.* 2015; 26:1013–1016.
- [33]. Bailey TS, Zakharov LN, Pluth MD. Understanding hydrogen sulfide storage: Probing conditions for sulfide release from hydrodisulfides. *J. Am. Chem. Soc.* 2014; 136:10573–10576. [PubMed: 25010540]
- [34]. Tsurugi J, Nakabayashi T. Synthesis of dibenzhydryl and dibenzyl penta- and hexasulfides. *J. Org. Chem.* 1959; 24:807–810.
- [35]. Heimer NE, Field L, Wailes JA. Organic disulfides and related substances. 44. Preparation and characterization of 1-adamantyl hydrodisulfide as a stable prototype of the series. *J. Org. Chem.* 1985; 50:4164–4166.
- [36]. Libiad M, Yadav PK, Vitvitsky V, Martinov M, Banerjee R. Organization of the human mitochondrial hydrogen sulfide oxidation pathway. *J. Biol. Chem.* 2014; 289:30901–30910. [PubMed: 25225291]
- [37]. Hall CD, Tweedy BR, Kayhanian R, Lloyd JR. The kinetics and mechanism of the reaction of tricoordinate phosphorus compounds with diaryl trisulfides. *J. Chem. Soc. Perkin Trans.* 1992; 2:775–779.
- [38]. Ahriks A, Robert J, Anouti M, Paris J. Nucleophilic substitution of alkyl halides by electrogenerated polysulfide ions in N,N-dimethylacetamide. *Acta Chem. Scand.* 1999; 53:513–520.
- [39]. Liu DK, Chang SG. Kinetic study of the reaction between cystine and sulfide in alkaline solutions. *Can. J. Chem.* 1987; 65:770–774.

Highlights

- Persulfides are biologically important molecules
- Persulfide reactivity remains underinvestigated
- Adamantyl and benzyl persulfides are prepared and fully characterized
- Persulfide reactivity is studied in non-polar anaerobic solvents
- A model for the reactivity of the studied persulfides is proposed

Figure 1.

Chemical structure of the prepared persulfides: trityl persulfide (TrtSSH), adamantyl persulfide (AdSSH), and benzyl persulfide (BnSSH).

Figure 2.

UV-Vis spectrum of 100 μM BnSSH reacting with 10 mM $[\text{NBu}_4^+][\text{AcO}^-]$ in dichloromethane. Immediately after addition of base (black), the intensity of the peak centered around 340 nm increases for 30 min (blue), before returning to background levels (red). Inset: Intensity of persulfide anion (340 nm) with respect to time.

General **Sterically-Hindered or Tertiary Persulfide** **Sterically-Accessible or Primary Persulfide** **Figure 3.**

Proposed reaction mechanism for the reaction of persulfides (RSSH) with reductants and bases.

Scheme 1.

General synthetic route to access organic persulfides.

Table 1

Spectroscopic properties for the ^1H NMR resonance (δ) and IR stretching frequency (ν) of the S–H bond for AdSSH, BnSSH, and TrtSSH compared with their parent thiols.

R	δ (ppm)		ν_{SH} (cm^{-1})	
	RSSH	RSH	RSSH	RSH
Trt	2.75	3.10	2508	2574
Bn	3.02	1.86	2504	2560
Ad	2.70	1.57	2491	2567

Table 2

Reactivity of persulfides in the presence of different reductants, nucleophiles, gases, and bases. Experiments were performed anhydrously and anaerobically in CD_2Cl_2 at room temperature with 18 mM persulfide and reactant. All reaction products were confirmed by ^1H or $^{31}\text{P}\{^1\text{H}\}$ NMR spectroscopy. S_8 formation was confirmed and quantified by titration with PPh_3 at the end of the reaction. Abbreviations: nr: no reaction, DTP: 1,3-dithiopropene. Reactions taking > 48 hours to occur are considered exhibit no reactivity.

Reactant	TrtSSH		BnSSH		AdSSH	
	Products	time	Products	time	Products	time
PPh_3	TrtSH, Ph_3PS	< 2 min	BnSH, BnSSSBn, H_2S , Ph_3PS	16 hr	AdSH, Ph_3PS	> 24 hr
DTP	Nr	–	nr	–	nr	–
$\text{H}_2\text{S}_{(\text{g})}$	Nr	–	nr	–	nr	–
$\text{NO}_{(\text{g})}$	Nr	–	nr	–	nr	–
$[\text{Na}^+][\text{TrtS}^-]$	TrtSH, S_8	< 2 min	Polysulfides	< 2 min	AdSH, S_8	< 2 min
$[\text{NBu}_4^+][\text{HS}^-]$	TrtSH, S_8 , H_2S	> 24 hr	H_2S , polysulfides	< 2 min	nr	–
$[\text{NBu}_4^+][\text{CN}^-]$	TrtSH, S_8	< 2 min	H_2S , BnSH, polysulfides	< 2 min	AdSH, S_8	< 2 min
$[\text{NBu}_4^+][\text{AcO}^-]$	TrtSH, S_8	< 2 min	Polysulfides	< 2 min	AdSH, S_8	< 2 min
$[\text{NBu}_4^+][\text{Cl}^-]$	TrtSH, S_8	1 hr	polysulfides	< 2 min	nr	–
$[\text{NBu}_4^+][\text{I}^-]$	nr	–	polysulfides	> 24 hr	nr	–
NEt_3	TrtSH, S_8	< 2 min	polysulfides	< 2 min	AdSH, S_8	30 min

Table 3

Distribution of products observed in the reactions of benzyl persulfide as measured by integration of the peaks present in the product spectra. The polysulfides were identified using their reported chemical shifts.[38]

Reactant	BnSH	BnSSBn	BnS ₃ Bn	BnS ₅ Bn	BnS ₆ Bn
PPh ₃	0.54	0.07	0.39	-	-
[Na ⁺][TrtS ⁻]	0.41	-	0.30	0.22	0.07
[NBu ₄ ⁺][HS ⁻]	-	-	0.58	0.33	0.09
[NBu ₄ ⁺][CN ⁻]	0.28	0.15	0.37	0.14	0.06
[NBu ₄ ⁺][AcO ⁻]	0.26	0.08	0.48	0.18	-
[NBu ₄ ⁺][Cl ⁻]	-	-	0.61	0.28	0.11
[NBu ₄ ⁺][I ⁻]	-	-	0.5	0.35	0.15
NEt ₃	0.28	0.03	0.39	0.21	0.09