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Generation of DNA-Damaging Reactive Oxygen Species via the Autoxidation of Hydrogen Sulfide under Physiologically Relevant Conditions: Chemistry Relevant to Both the Genotoxic and Cell Signaling Properties of H_2S

Marjorie Hoffman,[†] Anuruddha Rajapakse,[†] Xiulong Shen,[†] and Kent S. Gates*[,†,‡]

[†]Department of Chemistry and [‡]Department of Biochemistry, University of Missouri, 125 Chemistry Building, Columbia, Missouri 65211, United States

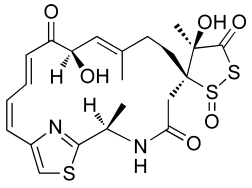
ABSTRACT: Hydrogen sulfide (H_2S) has long been known for its toxic properties; however, in recent years, evidence has emerged that this small, gaseous molecule may serve as an endogenous cell-signaling agent. Though perhaps surprising in light of its potential role as an endogenous signaling agent, a number of studies have provided evidence that H_2S is a DNA-damaging mutagen. In the work reported here, the chemical mechanisms of DNA damage by H_2S were examined. Using a plasmid-based DNA strand cleavage assay, we found that micromolar concentrations of H_2S generated single-strand DNA cleavage. Mechanistic studies indicate that this process involved autoxidation of H_2S to generate superoxide, hydrogen peroxide, and, ultimately, the well-known DNA-damaging agent hydroxyl radical via a trace metal-mediated Fenton-type reaction. Strand cleavage by H_2S proceeded in the presence of physiological thiol concentrations, and the known byproducts of H_2S oxidation such as thiosulfate, sulfite, and sulfate do not contribute to the strand cleavage process. However, initially generated oxidation products such as persulfide (S_2^{2-}) likely undergo rapid autoxidation reactions that contribute to the generation of superoxide. The potential relevance of autoxidation processes to the genotoxic and cell signaling properties of H_2S is discussed.

$$H_2S \rightleftharpoons HS^- + H^+ \qquad HS^- + M^{(n+1)+} \rightarrow HS\bullet + M^{n+} \qquad M^{n+} + O_2 \rightarrow M^{(n+1)+} + O_2\bullet^-$$

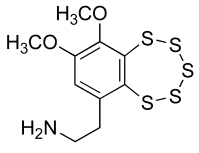
$$O_2\bullet^- + HO_2\bullet \rightarrow H_2O_2 + O_2 \qquad H_2O_2 + M^{n+} \rightarrow HO\bullet + HO^- + M^{(n+1)+} \qquad HO\bullet + \text{H-DNA} \rightarrow HOH + \bullet\text{DNA}$$

INTRODUCTION

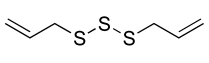
Hydrogen sulfide (H_2S) has long been known for its toxic properties;[1−5] however, in recent years, evidence has emerged that this small molecule may serve as a cell signaling agent in mammals.[6−13] H_2S has been implicated in the modulation of diverse processes including inflammation,[14] angiogenesis,[15] cytoprotection,[16] nociception,[17] stimulation of ATP-sensitive potassium ion channels,[18] myocardial contractility,[19] and vascular tone and blood pressure.[20,21] Some sulfur-containing small molecules including leinamycin,[22] 1,2-dithiolan-3-ones,[23] polysulfides,[22,24−26] varacin,[22,24−26] lissoclinotoxin A,[22,24−26] 3*H*-1,2-dithiole-3-thiones,[27,28] and garlic-derived phytochemicals such as *S*-allylcysteine,[29,30] allicin,[29,30] diallyl disulfide,[21,31] and diallyl trisulfide[21] may gain at least a portion of their bioactivities via the release of H_2S.



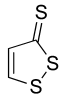
Leinamycin



Varacin



Diallyl trisulfide



3*H*-1,2-Dithiole-3-thione

Though perhaps surprising in light of its potential role as an endogenous signaling agent, there is evidence that H_2S is a DNA-damaging mutagen. For example, H_2S showed genotoxicity in a modified comet assay where DNA repair was inhibited[32] and in nontransformed human intestinal epithelial cells.[33] In naked nuclei of Chinese hamster ovary cells, H_2S caused nucleobase damage that was excised by the repair enzyme formamidopyrimidine glycosylase (FPG).[34] Evidence for the oxidative nature of this base damage was inferred from the observation that the radical scavenger *t*-butylhydroxyanisole inhibited its formation. In a separate study, H_2S was found to cause increased levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine in coelomocytes and in *Glycera dibranchiata*.[35] In cultured human lung fibroblasts, H_2S induced a concentration-dependent increase in micronuclei, a finding suggestive of DNA damage.[36] Finally, H_2S was shown to be weakly mutagenic in the *Salmonella typhimurium* strain 1535.[37] Transition metal-dependent autoxidation of H_2S in the environment has been characterized,[38,39] but the generation of DNA-damaging reactive oxygen species by these processes under physiologically relevant conditions has not been well studied. In the work reported here, the chemical mechanisms of DNA damage initiated by the autoxidation of H_2S under physiological conditions were examined.

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EXPERIMENTAL PROCEDURES

Caution: *H_2S is highly toxic. Exposure to this gas can be fatal. Appropriate precautions must be taken when working with H_2S gas and aqueous solutions containing the salts Na_2S and NaSH.*[41]

Materials. Reagents were purchased from the following suppliers and were of the highest purity available: sodium phosphate, ethidium bromide, mannitol, 2-mercaptoethanol, L-cysteine, dithiothreitol, and sodium bisulfite from Aldrich Chemical Co.; sodium sulfide nonahydrate, NaSH·$x$$H_2O$, H_2S gas, superoxide dismutase (SOD), catalase, glutathione, sodium thiosulfate pentahydrate, and Tris-HCl, EDTA from Sigma Chemical Co.; water (HPLC grade), sodium sulfite, and sodium sulfate from Fisher Scientific; absolute ethanol from Decon Laboratories; diethylenetriaminepentaacetic acid (DETAPAC) from Fluka; and agarose from Lonza. Before use, the sodium sulfide was rinsed with distilled, deionized water to remove oxide impurities from the surface and then dried as described previously (weighing of freshly washed, damp material may lead to a slight underestimation of stock concentrations).[40]

Cleavage of Plasmid DNA by H_2S. In a typical assay, supercoiled double-stranded plasmid DNA (pGL2-Basic, 1 μL of a 1 mg/mL solution in 10 mM Tris-HCl, and 1 mM EDTA, pH 7.5) was added to water (17 μL), followed by the addition of sodium sulfide nonahydrate (Na_2S, 2 μL in sodium phosphate, pH 7, 500 mM). The solution was gently mixed, spun for 2 s in a tabletop centrifuge, and incubated at 37 °C for 12−14 h. Solutions of sodium sulfide were prepared immediately before use and used within 5 min of preparation. In mechanistic experiments where various additives were present in the assays, these agents were placed in the reaction mixture prior to the addition of DNA. After incubation, loading buffer (5 μL of a 50% glycerol loading buffer[42]) was added, and the reaction mixtures were loaded onto a 0.9% agarose gel. The gel was electrophoresed for 2−3 h at 80 V in 1× TAE buffer and then stained in a dilute solution of aqueous ethidium bromide. The gel was placed on a UV transilluminator and the amount of DNA in each band quantified using an Alpha Innotech IS-1000 digital imaging system. Solutions of NaSH were calculated using a molecular weight of 56.1 g/mol. Because the NaSH salt contains waters of hydration (usually about 2−3), the reported concentrations are somewhat higher than the actual concentrations.

RESULTS

DNA Strand Cleavage by H_2S. H_2S can be introduced into aqueous solutions as the gaseous form or as the salts Na_2S or NaSH.[41] Regardless of the form in which it is introduced into solution, at pH 7.4 and 25 °C, an equilibrium mixture composed of H_2S (~30%) and the monoanion HS^- (~70%) is established, with the dianion S^{2-} present in very small amounts (for H_2S, pK_{a1} = 6.98; pK_{a2} ~19).[41] Here, we refer to this collective equilibrium mixture as "H_2S". Sulfur anions readily undergo trace metal-mediated oxidation in aerobic solution to generate the superoxide radical ($O_2^{\cdot-}$, eqs 1−3).[26,38,43−47] Superoxide radical disproportionates to yield hydrogen peroxide (H_2O_2), which, in turn, can undergo a Fenton-type reaction involving adventitious traces of transition metals to yield the well-known DNA strand-cleaving agent hydroxyl radical (HO·, eqs 4 and 5).[48] Accordingly, we examined the activity of H_2S in a plasmid-based assay that readily measures strand cleavage by reactive oxygen species (ROS).[22,24,26] In this assay, single-strand cleavage converts supercoiled plasmid DNA (form I) to the open-circular form (form II).[49−51] The two forms of plasmid DNA are then separated using agarose gel electrophoresis, the gel stained with a DNA-binding dye such as ethidium bromide and the relative amounts of cleaved and intact plasmid quantitatively determined by digital image analysis. Direct strand breaks (not requiring thermal or basic workup) monitored in this type of experiment typically arise via the reaction of radicals with hydrogen atoms on 2′-deoxyribose residues in the backbone of DNA.[52−55]

Using Na_2S as a source of H_2S, we observed concentration-dependent cleavage of duplex DNA (Figure 1). At Na_2S concentrations in the range of 10−1000 μM, nicked (form II) plasmid resulting from single-strand cleavage was observed, while no linearized (form III) plasmid arising from double-strand breakage was seen. Significant strand cleavage was observed at Na_2S concentrations as low as 10 μM. The data suggests a nonlinear increase in the yields of DNA strand breaks with increasing Na_2S concentration in the range 10−1000 μM (Figure 1B). Previous reports indicate that high H_2S:O_2 ratios favor the formation of elemental sulfur as an oxidation product.[44,56] Elemental sulfur may react with HS^- to generate polysulfides S_n^{2-} that, in turn, react with O_2 to generate additional superoxide radicals.[44,56−58] To probe this possibility directly, we investigated the effect of added elemental sulfur (S_8, added as a suspension in water) on DNA cleavage by Na_2S. These reactions were carried out under our standard reaction conditions reported in the legend of Figure 1, except at 24 °C. S_8 alone (16 nM) generated 0.44 ±

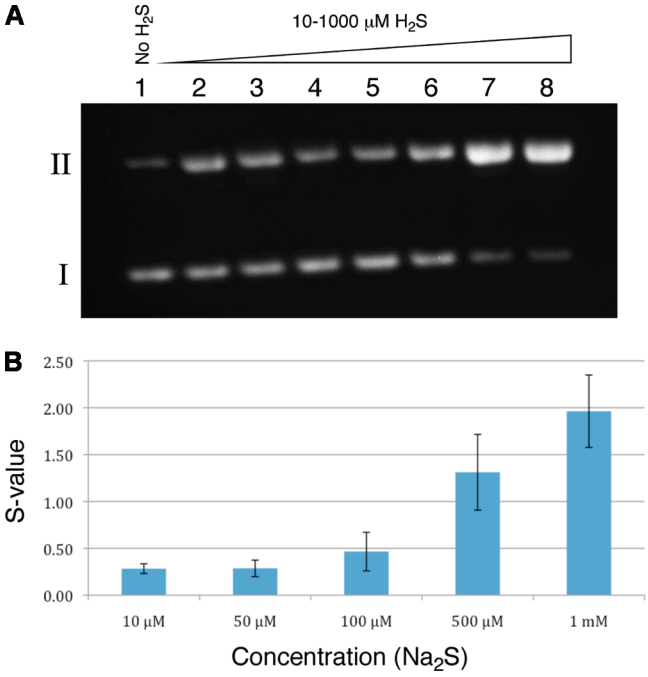


Figure 1. DNA strand cleavage by H_2S. Strand cleavage assays were performed as described in Experimental Procedures. Briefly, supercoiled double-stranded plasmid DNA (pGL2-Basic, 1 μL of a 1 mg/mL solution in 10 mM Tris-HCl and 1 mM EDTA, pH 7.5) was added to water (17 μL), followed by the addition of sodium sulfide nonahydrate (2 μL in sodium phosphate, pH 7, 500 mM). After12 h of incubation, forms I and II plasmid DNA were resolved on an agarose gel and stained with ethidium bromide. The DNA was visualized by UV-transillumination and the amounts measured by digital image analysis. The number of single strand breaks per plasmid DNA molecule (S) was calculated using the equation S = −ln f_I, where f_I is the fraction of plasmid present as form I.[97] (A) Treatment of plasmid DNA with H_2S led to an increase in the amount of cleaved, form II DNA. Lane 1 contained plasmid with no H_2S, while lanes 2−8 contained 10, 25, 50, 100, 250, 500, and 1000 μM H_2S, respectively. (B) A plot of strand cleavage yields (S) versus H_2S concentration. Here, the yields of strand cleavage were corrected for the amount of form II plasmid present in untreated plasmid DNA (lane 1).

0.20 strand breaks above background, comparable to the 0.41 ± 0.11 strand breaks generated by Na_2S (250 μM) alone. The combination of S_8 (16 nM) and Na_2S gave a synergistic increase to yield 1.98 ± 0.10 strand breaks above background.

As part of this work, we compared strand cleavage by Na_2S to that by NaSH. Interestingly, at higher concentrations (500−1000 μM) the yields of strand cleavage engendered by NaSH were significantly greater than that observed for Na_2S. For example, at concentrations of 500 μM, Na_2S and NaSH gave 1.3 ± 0.4 and 2.1 ± 0.4 strand breaks per plasmid, respectively, under the standard reaction conditions described in the legend of Figure 1. This may reflect the action of polysulfide contaminants such as S_3^{2-} that are commonly present in the ($NaSH{\cdot}xH_2O$) reagent.[41] Differences in the properties of Na_2S and NaSH may be noteworthy in light of the widespread use of NaSH as a source of H_2S in biological studies.

Mechanism of Strand Cleavage by H_2S. As noted above, trace metal-mediated autoxidation of HS^- may generate $O_2{\cdot}^-$.[26,38,43−47] To probe the involvement of $O_2{\cdot}^-$, H_2O_2, HO·, and adventitious transition metals in strand cleavage by H_2S, we performed a series of cleavage assays in the presence of additives that interact with various species shown in eqs 2−5.[48] For example, we found that strand cleavage was inhibited by the classical hydroxyl radical scavengers[48] methanol, ethanol, and DMSO (Table 1). The hydrogen peroxide-destroying enzyme catalase also inhibited strand cleavage.

Table 1. Effect of Additives on DNA Cleavage by H_2S[a]

reaction/additive	% nicked, form II DNA	S-value
DNA alone	32.2	0.39 ± 0.04
250 μM Na_2S (Std.)	49.8	0.70 ± 0.09
Std. + methanol (500 mM)	33.1	0.40 ± 0.06
Std. + ethanol (500 mM)	31.4	0.38 ± 0.05
Std. + mannitol (100 mM)	32.8	0.40 ± 0.04
Std. + DETAPAC (10 mM)	29.8	0.36 ± 0.03
Std. + SOD (100 μg/mL)	88.2	2.18 ± 0.23
Std. + catalase (100 μg/mL)	42.4	0.55 ± 0.03

[a]Strand cleavage assays were performed as described in Experimental Procedures. Briefly, supercoiled double-stranded plasmid DNA (pGL2-Basic, 1 μL of a 1 mg/mL solution in 10 mM Tris-HCl and 1 mM EDTA, pH 7.5) was added to water (17 μL), followed by the addition of sodium sulfide nonahydrate (2 μL in sodium phosphate, pH 7, 500 mM). Forms I and II plasmid DNA were resolved on an agarose gel and stained with ethidium bromide. The DNA was visualized by UV-transillumination and the amounts measured by digital image analysis. The number of single strand breaks per plasmid DNA molecule (S) was calculated using the equation $S = -\ln f_1$ where f_1 is the fraction of plasmid present as form I.[97]

$$H_2S \rightleftharpoons HS^- + H^+ \quad (1)$$

$$HS^- + M^{(n+1)+} \rightarrow HS{\cdot} + M^{n+} \quad (2)$$

$$M^{n+} + O_2 \rightarrow M^{(n+1)+} + O_2{\cdot}^- \quad (3)$$

$$O_2{\cdot}^- + HO_2{\cdot} \rightarrow H_2O_2 + O_2 \quad (4)$$

$$H_2O_2 + M^{n+} \rightarrow HO{\cdot} + HO^- + M^{(n+1)+} \quad (5)$$

$$HS^- + O_2 \xrightarrow{SOD} S^o + H_2O_2 \quad (6)$$

Interestingly, the addition of superoxide dismutase (SOD) significantly *increased* the yield of DNA strand breaks. There are at least two possible reasons for this effect, both of which are consistent with the reaction cascade shown in eqs 1−5. First, SOD catalyzes the disproportionation of $O_2{\cdot}^-$ to H_2O_2 and O_2.[48] Although spontaneous disproportionation of $O_2{\cdot}^-$ is fast,[48,59] the ability of SOD to accelerate this reaction nonetheless has the potential to increase the yield of H_2O_2 formation, thus increasing the yields of strand cleavage stemming from the reactions shown in eqs 1−5. Second, and likely more important, SOD acts as an HS^-:O_2 oxidoreductase that converts HS^- and O_2 into H_2O_2 and S^o (eq 6, where S^o is defined as elemental sulfur and related "sulfane" species in which sulfur is bonded only to sulfur).[60] Under the reaction conditions employed here, the resulting elemental sulfur is expected[25,61] to react with HS^- to generate polysulfides (S_n^{2-}) that can react with O_2 to generate additional superoxide radical via reactions analogous to those shown in eqs 2 and 3. Indeed, we presented evidence above showing that the addition of elemental sulfur to a standard Na_2S reaction significantly increased strand cleavage.

DNA cleavage also was effectively suppressed by the presence of diethylenetriaminepentaacetic acid (DETAPAC), a chelator of adventitious metals that inhibits transition metal-dependent Fenton-type reactions (eq 5).[48] In analogy with the ability of chelators to inhibit metal-mediated oxidation of organic thiolates (RS^-),[45] it also was expected that DETAPAC could inhibit the initial metal-mediated oxidation of HS^- (eq 2).[38] To provide further evidence for the role of metals in the H_2S-mediated strand cleavage process, we carried out a complementary experiment in which we added small amounts of the transition metal Fe(III) to the reaction mixtures. We found that Fe(III) concentrations between 1 nM and 1 μM significantly increased the strand cleavage induced by H_2S (Table 2). Fe(III) at these low concentrations did not induce significant strand cleavage on its own. These results confirmed the metal-dependence of the H_2S-mediated strand cleavage process.

Investigating Strand Cleavage by the H_2S Decomposition Products Thiosulfate, Sulfite, and Sulfate. H_2S readily undergoes oxidation both in aerobic solution and in

Table 2. Cleavage of Plasmid DNA by H_2S in the Presence of Various Concentrations of Iron[a]

reaction/additive	% nicked, form II DNA	S-value
DNA alone	33.9	0.40 ± 0.05
250 μM Na_2S alone (Std.)	48.2	0.60 ± 0.04
Std. + FeSO4 (1 nM)	53.7	0.80 ± 0.14
Std. + FeSO4 (100 nM)	66.3	1.01 ± 0.06
Std. + FeSO4 (1 μM)	83.9	1.83 ± 0.04
FeSO4 alone (1 μM)	32.1	0.36 ± 0.07

[a]Strand cleavage assays were performed as described in Experimental Procedures. Briefly, supercoiled double-stranded plasmid DNA (pGL2-Basic, 1 μL of a 1 mg/mL solution in 10 mM Tris-HCl and 1 mM EDTA, pH 7.5) was added to water (17 μL), followed by the addition of sodium sulfide nonahydrate (2 μL in sodium phosphate, pH 7, 500 mM). Forms I and II plasmid DNA were resolved on an agarose gel and stained with ethidium bromide. The DNA was visualized by UV-transillumination and the amounts measured by digital image analysis. The number of single strand breaks per plasmid DNA molecule (S) was calculated using the equation $S = -\ln f_1$, where f_1 is the fraction of plasmid present as form I.[97]

cells.[6,41,62−65] The products of this oxidation process include thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}), and sulfate (SO_4^{2-}).[6,41,66] Given that sulfite and bisulfite (HSO_3^-, the protonated form of sulfite), at least, have been reported to undergo metal-mediated autoxidation reactions that generate reactive oxygen and sulfur species,[67−69] we felt it was important to investigate the ability of various H_2S oxidation products to cause strand cleavage under the conditions of our assay. In the event, we found that none of these H_2S oxidation products generated significant levels of strand cleavage. Overall, the data indicate that the expected H_2S oxidation products thiosulfate, sulfite, and sulfate do not contribute to the DNA strand cleavage observed under our reaction conditions.

Effect of Thiols on DNA Strand Cleavage by H_2S. Cells contain millimolar concentrations of thiols such as glutathione.[70−72] Therefore, we examined the effects of added thiols on the ability of H_2S to cause DNA strand cleavage. We find that Na_2S (1 mM) in the presence of 2-mercaptoethanol (10 mM) yields 1.2 ± 0.3 strand breaks above background (Table 3). Under the same conditions, except in the absence of thiol, Na_2S generates 1.9 ± 0.4 strand breaks. The compound 2-mercaptoethanol alone (10 mM) generates 0.6 ± 0.5 strand breaks above the background levels of strand breaks present in the plasmid substrate, under the reaction conditions employed here. The result of this control reaction is consistent with previous reports indicating that thiols alone generate DNA strand breaks via autoxidation processes that produce ROS.[73,74] Overall, our results provide evidence that H_2S generates DNA strand cleavage in the presence of thiols, although the cleavage yields are somewhat diminished. Similar results were observed at lower concentrations of Na_2S and thiol and with the biological thiol, glutathione. In general, thiols have the potential to act as either prooxidants or antioxidants. When incubated alone in the plasmid-based DNA-cleavage assay, the mild prooxidant properties of 2-mercaptoethanol are displayed. However, with respect to the strand cleavage caused by Na_2S, added thiol appears to serve as an antioxidant.

Table 3. Cleavage of Plasmid DNA by H_2S Oxidation Products[a]

reaction/additive	% nicked, form II DNA	S-value
DNA alone	31.8	0.33 ± 0.02
500 μM Na_2S	43.2	0.56 ± 0.08
1 mM Na_2S	46.3	0.63 ± 0.12
500 μM NaS_2O_3	27.8	0.30 ± 0.02
1 mM NaS_2O_3	28.1	0.30 ± 0.02
500 μM Na_2SO_3	28.3	0.31 ± 0.04
1 mM Na_2SO_3	27.8	0.29 ± 0.03
500 μM Na_2SO_4	23.7	0.23 ± 0.02
1 mM Na_2SO_4	24.7	0.23 ± 0.04
500 μM $NaHSO_3$	29.8	0.28 ± 0.00
1 mM $NaHSO_3$	31.4	0.31 ± 0.03

[a]Strand cleavage assays were performed as described in Experimental Procedures. Briefly, supercoiled double-stranded plasmid DNA (pGL2-Basic, 1 μL of a 1 mg/mL solution in 10 mM Tris-HCl and 1 mM EDTA, pH 7.5) was added to water (17 μL), followed by the addition of sodium sulfide nonahydrate (2 μL in sodium phosphate, pH 7, 500 mM). Forms I and II plasmid DNA were resolved on an agarose gel and stained with ethidium bromide. The DNA was visualized by UV-transillumination and the amounts measured by digital image analysis. The number of single strand breaks per plasmid DNA molecule (S) was calculated using the equation $S = -\ln f_1$, where f_1 is the fraction of plasmid present as form I.[97]

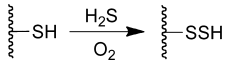
DISCUSSION

Our results indicate that H_2S undergoes trace metal-mediated autoxidation to generate superoxide, hydrogen peroxide, and, ultimately, the well-known DNA-cleaving agent hydroxyl radical. The metal dependence of this process is consistent with previous reports regarding the role of transition metals in the environmental oxidation of sulfide.[38,39] Though cells contain little or no free transition metals, it is clear that protein-bound metals are capable of participating in such redox processes.[48] Indeed, hemeprotein-mediated autoxidation processes have been suggested to contribute to the cytotoxicity of H_2S.[4] It may be significant that, at physiological pH, significant amounts of H_2S exist as the sulfur anion HS^-, which is the principal substrate for aerobic oxidation.[41,43] In contrast, typical pK_a values for thiols are substantially higher (e.g., the pK_a of the thiol group in cysteine is 8.3), and relatively small amounts of the thiolate anions (RS^-) are present.

Our results showed that the H_2S oxidation products thiosulfate, sulfite, and sulfate do not contribute to the strand cleavage processes examined here. However, initially generated oxidation products such as persulfide (S_2^{2-}) likely undergo rapid autoxidation reactions that contribute to the generation of superoxide under our reaction conditions.[44,56−58] The nonlinear increase in DNA strand cleavage with increasing H_2S concentrations may mesh with previous reports indicating that high H_2S:O_2 ratios favor the formation of elemental sulfur as an oxidation product.[44,56] Elemental sulfur may react with HS^- to generate polysulfides S_n^{2-} that, in turn, react with O_2 to generate additional superoxide radical.[44,56−58] In this manner, polysulfides act as catalysts for sulfide oxidation and the concomitant production of ROS.[22,75] Indeed, we provided evidence here that addition of even small amounts of elemental sulfur dramatically increased the DNA-cleaving properties of Na_2S. Finally, we showed that DNA strand cleavage by H_2S proceeded in the presence of physiological thiol concentrations.

Redox chemistry of the type described here could underlie much of the biological activity associated with H_2S. For example, the ability of H_2S to generate superoxide, hydrogen peroxide, and hydroxyl radical under physiological conditions may explain the mutagenic properties of H_2S.[32,34−37] Hydroxyl radical is a well-characterized mutagen.[52,76] Furthermore, H_2O_2 produced in the autoxidation of H_2S may be relevant to the putative cell signaling properties of H_2S, given that H_2O_2 has recently become established as a cell signaling agent in its own right.[77−83] Thus, it is possible that, under some circumstances, H_2S serves as a means for generating H_2O_2 in cell signaling processes. Our results further highlight a potential role for the enzyme superoxide dismutase in catalyzing the generation of H_2O_2 from H_2S.[60] The production of ROS (specifically H_2O_2) may explain the ability of H_2S to activate the transcription factors such as Nrf2.[16,84] Likewise, H_2S could contribute to the activation of Nrf2 by agents such as 3*H*-1,2-dithiole-3-thiones and diallyl sulfides.[21,85−90] The oxidation of H_2S in the presence of protein thiols has the potential to generate protein persulfides (Scheme 1). It has been suggested that such protein sulfhydration reactions are involved in the cell signaling properties of H_2S.[7,91,92] Finally, it has been proposed that sulfane byproducts of H_2S oxidation (e.g., S_n^{2-} and S_8) may be the actual regulatory agents generated by H_2S.[93,94]

Scheme 1



Our work highlights some similarities between the cell signaling agents nitric oxide, H_2O_2, and H_2S. Each of these species can mediate controlled biological responses via the selective reactions with specific target proteins, yet also have potential to cause toxicity via "off target" reactions with bystander proteins and nucleic acids.[81,95,96] The DNA-damaging properties of H_2S discussed here and elsewhere[32−37] emphasize the importance of spatial control in the generation of H_2S if this agent does indeed serve as a cell signaling agent.

AUTHOR INFORMATION

Corresponding Author

*Phone: (573) 882-6763. Fax: (573) 882-2754. E-mail: gatesk@missouri.edu.

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Notes

The contents of this work are solely the responsibility of the authors and do not necessarily represent the official views of the NCCAM, ODS, NCI, or the National Institutes of Health. The authors declare no competing financial interest.

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