

A Sweet H₂S/H₂O₂ Dual Release System and Specific Protein S-Persulfidation Mediated by Thioglucose/Glucose Oxidase

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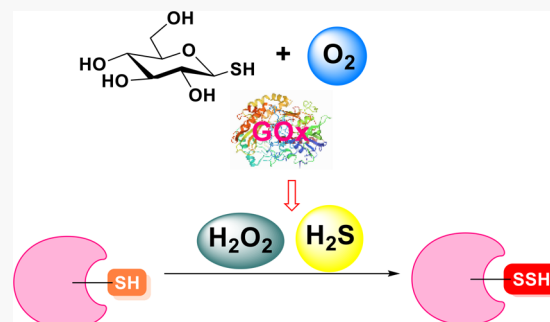


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ABSTRACT: H₂S and H₂O₂ are two redox regulating molecules that play important roles in many physiological and pathological processes. While each of them has distinct biosynthetic pathways and signaling mechanisms, the crosstalk between these two species is also known to cause critical biological responses such as protein S-persulfidation. So far, many chemical tools for the studies of H₂S and H₂O₂ have been developed, such as the donors and sensors for H₂S and H₂O₂. However, these tools are normally targeting single species (e.g., only H₂S or only H₂O₂). As such, the crosstalk and synergetic effects between H₂S and H₂O₂ have hardly been studied with those tools. In this work, we report a unique H₂S/H₂O₂ dual donor system by employing 1-thio-β-D-glucose and glucose oxidase (GOx) as the substrates. This enzymatic system can simultaneously produce H₂S and H₂O₂ in a slow and controllable fashion, without generating any bio-unfriendly byproducts. This system was demonstrated to cause efficient S-persulfidation on proteins. In addition, we expanded the system to thiolactose and thioglucose-disulfide; therefore, additional factors (β-galactosidase and cellular reductants) could be introduced to further control the release of H₂S/H₂O₂. This dual release system should be useful for future research on H₂S and H₂O₂.



INTRODUCTION

Reactive sulfur species (RSS) and reactive oxygen species (ROS) are two groups of molecules that play regulatory roles in redox biology. As the most well studied RSS, H₂S is endogenously produced by enzymes including cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and 3-mercaptopyruvate sulfur transferase (3-MST). Dysregulated H₂S levels are associated with pathological processes like cancer, inflammation, hypertension, etc.^{1,2} As the center species of ROS, H₂O₂ is formed from spontaneous dismutation of superoxide (O₂^{•-}) or under the catalysis of superoxide dismutase (SOD). Endogenous H₂O₂ can also be produced by oxidases, such as NADP/H oxidases (NOX), lysyl oxidases (LOX), xanthine oxidase (XO), amine oxidase (AO), etc.^{3,4} Aberrant production of H₂O₂ leads to oxidative stress and damage, which is connected to aging and neurodegenerative diseases.^{5,6} While H₂S and H₂O₂ have their very distinct signaling pathways, they also interact with each other and work collectively in redox signaling.⁷ The direct reaction between H₂S and H₂O₂ under physiological conditions is known to be slow, so their crosstalk is believed to work through indirect effects on enzymes and/or other targets in signaling pathways.⁷ For example, protein S-persulfidation is an important posttranslational modification regulated by H₂S. However, H₂S cannot directly react with protein thiols to form persulfides and its reaction with disulfides is normally slow and thermodynamically unfavored.^{8,9} It is likely that persulfidation is the result of the reaction between H₂S and

sulfenic acid (S–OH), which is the oxidation product from H₂O₂.⁹ As such, an appropriate level of H₂O₂ is required to facilitate H₂S signaling cascades, especially under oxidative stress. In another study H₂S was found to significantly amplify H₂O₂-based therapeutic treatment for cancer in a mouse model.¹⁰ Moreover, the mechanism of acute H₂S intoxication is due to H₂S serving as a substrate for complex II of the mitochondrial electron transport chain, thereby inducing high levels of ROS formation and oxidative stress.¹¹ These results indicate that concurrent presence of H₂S and H₂O₂ is often needed to reveal their true biological importance.

So far, many chemical tools have been developed for RSS/ROS studies. In particular, donor molecules for these reactive species have received considerable attention. For example, a variety of H₂S donors have been reported and they can be triggered by different cellular factors to release H₂S.^{12–14} Figure 1 shows a few examples: GYY4137 and JK-1 are pH-triggered donors. N-Benzoylthiobenzamides are thiol-triggered donors. A few enzyme-triggered donors are also reported, including the esterase-activated donor (HP-101), esterase and carbonic

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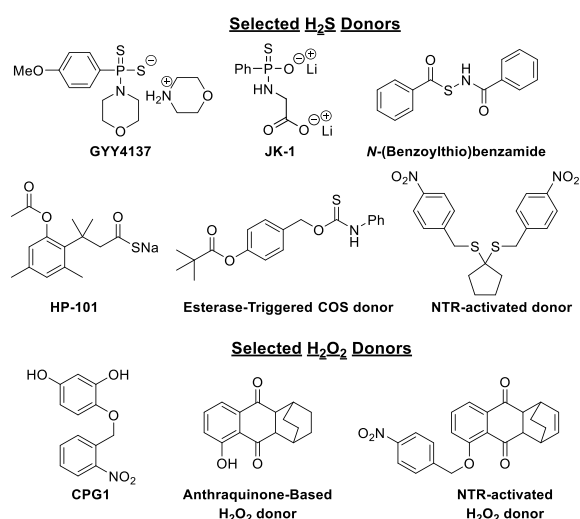


Figure 1. Representative examples of synthetic H₂S and H₂O₂ donors.

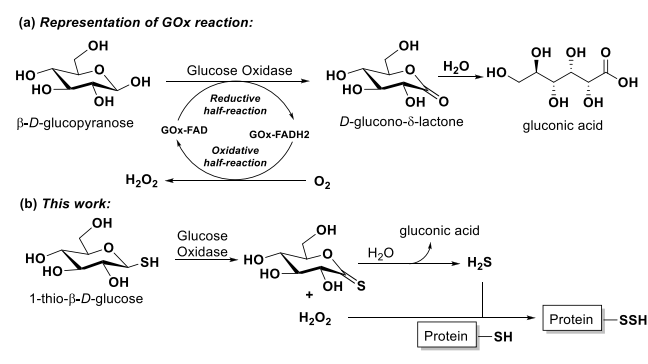
anhydrase (CA)-triggered COS/H₂S donors, and nitro-reductase-activated donors.^{15–17} H₂O₂ donors are much less developed, and researchers still tend to use H₂O₂ directly in studies. However, it has been demonstrated that bolus delivery of H₂O₂ is often problematic due to rapid consumption of H₂O₂ in biological systems such as in cells.^{18,19} This justifies the need for slow and continuous H₂O₂ releasing methods. Currently only a few hydroquinone and anthraquinone derivatives are reported as H₂O₂ donors (Figure 1).^{20–22}

While these donor compounds have advanced our understanding of H₂S and H₂O₂, limitations still remain: (1) All these donors are synthetic materials. Inevitably, they will also produce significant amounts of organic byproducts (in addition to H₂S or H₂O₂). This could cause unwanted side effects. (2) Enzyme-triggered donors (especially H₂O₂ donors) are still very limited. (3) All available donors can only produce one species (H₂S or H₂O₂), which can hardly mimic the concurrent presence of H₂S and H₂O₂ or allow the study of their crosstalk and synergetic effects. Herein, we would like to report a new H₂S/H₂O₂ dual donor system employing thioglucose and GOx. This can produce H₂S and H₂O₂ simultaneously in a slow and controllable fashion, without generating any bio-unfriendly organic byproducts. We also demonstrated that this system can cause efficient S-persulfidation on proteins. Moreover, this system can be expanded to the disulfides of thioglucose and thiolactose. As such, additional factors can be induced to further control the release of H₂S/H₂O₂ dual species.

RESULTS AND DISCUSSION

Glucose oxidase (GOx) is a flavin-containing oxido-reductase which catalyzes the oxidation of β-D-glucose to D-glucono-δ-lactone by O₂ gas with the production of H₂O₂ (Scheme 1a).²³ Because of this property, the glucose/GOx system has been used as an alternative for the exogenous addition of H₂O₂ in biological studies.¹⁹ This system can induce hypoxia, oxidation stress, or enhance acidity in tumor microenvironments. These have been strategically utilized to achieve multimodal synergistic cancer therapy by integrating GOx with various therapeutic approaches.²⁴ From a mechanistic point-of-view, if 1-thio-β-D-glucose (thioglucose)²⁵ is used as the substrate for GOx, it would produce thio-gluconolactone as the product,

Scheme 1. Idea of Using Thioglucose/GOx to Release H₂S/H₂O₂ and Induce Protein S-Persulfidation



which should easily undergo hydrolysis to release H₂S. Therefore, we envisioned that thioglucose/GOx could be a unique enzyme triggered H₂S/H₂O₂ dual releasing system (Scheme 1b). This is attractive, as only nontoxic and biocompatible gluconic acid would accompany the two species. We also envisioned that the dual release of H₂S/H₂O₂ might be an efficient way to induce protein S-persulfidation. Furthermore, the thioglucose moiety can be engineered as stimuli-response prodrugs to deliver H₂S and H₂O₂ upon specific biologically relevant activation.

With this idea in mind, we first evaluated H₂S release from thioglucose in the presence of GOx. The standard methylene blue (MB) method was used to quantify H₂S production. As shown in Figure 2, thioglucose itself was found to be stable in

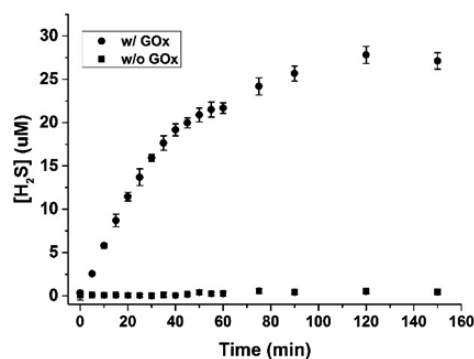


Figure 2. H₂S release profile of thioglucose (100 μM) in the presence and absence of GOx (10 μg/mL) in PBS buffer (50 mM, pH 7.4). The experiments were performed in triplicate, and results are expressed as mean ± SD (*n* = 3).

buffers and no obvious H₂S release was detected in the absence of GOx. However, significant H₂S release was observed when GOx was present. The optimized condition was identified as 100 μM thioglucose in PBS buffer (50 mM, pH 7.4) containing 10 μg/mL GOx. This combination led to slow, continuous, and time-dependent H₂S generation. The concentration of H₂S reached the maximum value of ~28 μM in about 2 h and then slowly decreased presumably due to volatilization of H₂S gas.

The H₂S formation was further verified by fluorescence measurements with the H₂S-specific probe WSP5.²⁶ As shown in Figure 3, the treatment of WSP5 with thioglucose or GOx alone did not give any detectable fluorescence. However, significant fluorescent signals were observed after incubating the probe (10 μM) with the mixture of thioglucose (100 μM)

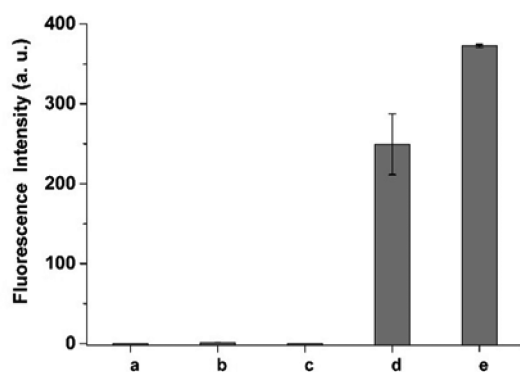


Figure 3. H₂S release from thioglucose (100 μM) in the presence of GOx (10 μg/mL) detected by WSP5 (10 μM) in PBS buffer (50 mM, pH 7.4). (a) WSP5 only, (b) WSP5 + thioglucose, (c) WSP5 + GOx, (d) WSP5 + thioglucose + GOx, (e) WSP5 + 100 μM Na₂S. The experiments were performed in triplicate, and results are expressed as mean ± SD (*n* = 3).

and GOx (10 μg/mL) at room temperature for 1 h, indicating that thioglucose indeed produced H₂S in the presence of GOx.

We next monitored the formation of H₂O₂ by the ferrous oxidation–xylenol orange method (FOX 1 assay) under the optimized conditions described above. As shown in Figure 4, a

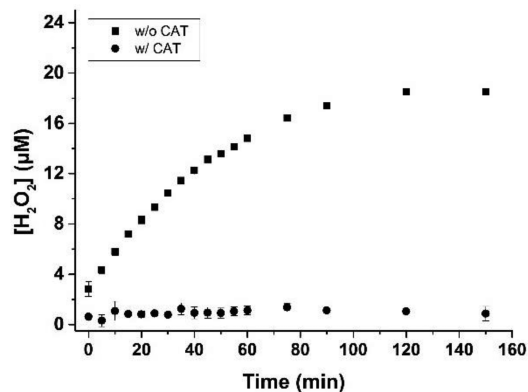


Figure 4. Time-dependent generation of H₂O₂ from thioglucose (100 μM) and GOx (10 μg/mL) in PBS buffer (50 mM, pH 7.4) in the presence and absence of CAT (50 μg/mL). The experiments were performed in triplicate, and results are expressed as mean ± SD (*n* = 3).

time-dependent H₂O₂ formation was observed with the peak concentration of ~20 μM at about 2 h. Additionally, if catalase (CAT) was present in this system (at 50 μg/mL), the produced H₂O₂ was completely scavenged. The H₂O₂ formation was also determined by fluorescence measurements with H₂O₂-sensitive Amplex Red/horseradish peroxidase (AP/HRP) assay (Figure S5). Interestingly, we noticed that the release of H₂S was not affected by the addition of CAT (Figure S2), indicating that thioglucose could be used as a clean H₂S donor if two enzymes (GOx and CAT) were applied.

The concurrent formation of H₂S and H₂O₂ in this system could lead to a concern that H₂S and H₂O₂ might react with each other, so the release of H₂S will not be efficient. However, the results shown in Figures 2 and 4 clearly suggested this should not present a major problem due to the slow reaction between them, especially under biologically relevant concentrations. It is reported that the second-order rate constant of

the reaction between H₂S and H₂O₂ is 0.73 M⁻¹ s⁻¹ (pH 7.4, 37 °C), with the formation of a mixture of hydrogen polysulfide (H₂S_{*n*}) as the possible products.²⁷ We also wondered if this system could produce H₂S_{*n*}. To test this, a specific fluorescent probe PSP-3²⁸ was used to monitor the generation of H₂S_{*n*}. As shown in Figure 5, negligible

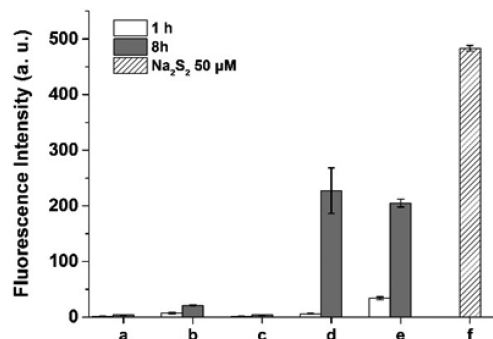


Figure 5. Detection of H₂S_{*n*} produced from thioglucose-GOx by PSP-3 (10 μM). Thioglucose (100 μM) was incubated with GOx (10 μg/mL) and PSP-3 in PBS buffer (50 mM, pH 7.4) for 1 h (white) and 8 h (gray), respectively. Fluorescence responses were recorded at 515 nm. (a) PSP-3 only, (b) PSP-3 + thioglucose, (c) PSP-3 + GOx, (d) PSP-3 + thioglucose + GOx, (e) PSP-3 + Na₂S₂ (100 μM) + H₂O₂ (100 μM), (f) PSP-3 + Na₂S₂ (50 μM). The experiments were performed in triplicate, and results are expressed as mean ± SD (*n* = 3).

fluorescence signals were observed after 1 h incubation of the probe with thioglucose (100 μM) and GOx (10 μg/mL) in PBS buffer (50 mM, pH 7.4) at rt. However, we did observe some fluorescence increases upon extending the incubation time to 8 h. We also compared this enzyme-generation system with directly mixing H₂O₂ and Na₂S. Weak fluorescence signals were observed with the incubation of a mixture of Na₂S (100 μM) and H₂O₂ (100 μM) after 1 h while much stronger fluorescence was noted after 8 h. These results again suggested that H₂S and H₂O₂ could react to form H₂S_{*n*} but requires a longer reaction time. This should not be a concern when the thioglucose-GOx system was used in our regular conditions (e.g., ~1 h).

Our results thus far have demonstrated that the thioglucose-GOx system under the optimized conditions could slowly and consistently produce H₂S and H₂O₂ under biologically relevant concentrations. We next wondered if this system could be used to induce protein S-persulfidation, an important posttranslational modification mediated by H₂S.^{9,29} This hypothesis was based on the knowledge that H₂S alone can hardly induce protein S-persulfidation (as the reaction between H₂S and protein disulfides is usually slow and less-productive). H₂O₂ can oxidize thiols in human serum albumin (HSA-SH) with a second-order rate constant of 2.3–2.7 M⁻¹ s⁻¹ (pH 7.4, 37 °C),³⁰ leading to the formation of sulfenic acid HSA-SOH that can be easily converted to persulfide upon reacting with H₂S. However, this method needs very high concentrations of H₂O₂ (4 mM) and H₂S (2 mM).³¹ We expected that our slow and continuous H₂S/H₂O₂ generation under low concentrations would have some advantages in inducing protein S-persulfidation. Before we tested this in proteins, we decided to test if thioglucose-GOx would induce S-persulfidation on low molecular weight (LMW) biothiols (such as Cys and GSH). SSP4, a persulfide sensitive fluorescent probe,³² was

used to measure persulfidation. Fluorescence intensities were measured after incubation of the probe with each analyte in PBS buffer (50 mM, pH 7.4) at rt. As shown in Figure 6, SSP-4

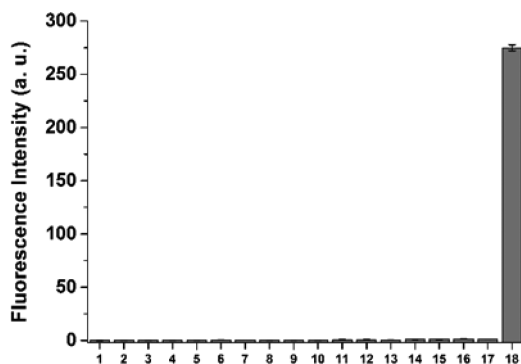


Figure 6. Fluorescence responses of SSP4 toward various low molecular weight biothiols in the presence of thioglucose-GOx. SSP4 (10 μ M) was incubated with different substrates in PBS buffer (50 mM, pH 7.4) for 1 h, then the fluorescence responses were recorded at 515 nm. (1) SSP4 only, (2) SSP4 + thioglucose (100 μ M), (3) SSP4 + GOx (10 μ g/mL), (4) SSP4 + Cys (100 μ M), (5) SSP4 + GSH (1 mM), (6) SSP4 + thioglucose (100 μ M) + Cys(100 μ M), (7) SSP4 + thioglucose (100 μ M) + GSH (1 mM), (8) SSP4 + GOx (10 μ g/mL) + Cys(100 μ M), (9) SSP4 + GOx (10 μ g/mL) + GSH(1 mM), (10) SSP4+ thioglucose (100 μ M) + GOx (10 μ g/mL), (11) SSP4 + Na₂S (30 μ M) + H₂O₂ (20 μ M), (12) SSP4+ thioglucose (100 μ M) + GOx (10 μ g/mL) + Cys(100 μ M), (13) SSP4+ thioglucose (100 μ M) + GOx (10 μ g/mL) + GSH(1 mM), (14) SSP4+ thioglucose (100 μ M) + Na₂S (30 μ M) + H₂O₂ (20 μ M), (15) SSP4 + GOx (10 μ g/mL)+ Na₂S (30 μ M) + H₂O₂ (20 μ M), (16) SSP4+ Cys(100 μ M) + Na₂S (30 μ M) + H₂O₂ (20 μ M), (17) SSP4 + GSH (1 mM) + Na₂S (30 μ M) + H₂O₂ (20 μ M), (18) SSP4 + Na₂S₂ (50 μ M). The experiments were performed in triplicate, and results are expressed as mean \pm SD ($n = 3$).

was quite stable upon treatment with a series of substrates alone including thioglucose, GOx, Cys, GSH, as well as the mixtures of thioglucose/Cys, thioglucose/GSH, GOx/Cys, GOx/GSH (columns 1–9). No fluorescence was noted in these studies. In addition, negligible responses were observed in the mixture of thioglucose/GOx (column 10) and the direct mixture of Na₂S/H₂O₂ under similar concentrations of the enzyme-promoted system (column 11). Furthermore, exposure of biothiols to thioglucose-GOx or the corresponding amounts of Na₂S/H₂O₂ showed no fluorescence increase (columns 12–17). As a positive control, SSP4 showed high fluorescence response to Na₂S₂ (column 18) a persulfide standard. These results clearly demonstrated that the thioglucose-GOx system does not induce effective persulfide formation on small molecular thiols.

Next, we tested if the combination of thioglucose and GOx could lead to the formation of protein persulfides. In this study, reduced BSA (30 μ M) was incubated with thioglucose (200 μ M) and GOx (10 μ g/mL) in PBS at room temperature for 1 h. CAT (25 μ g/mL) was then added to remove excess H₂O₂. SSP4 (10 μ M) was next applied to measure persulfide formation.³³ As demonstrated in Figure 7, this treatment (column h) led to an obvious increase in fluorescence, indicating the desired persulfide formation on BSA. Control experiments, e.g. BSA treated with each individual reagent used in the study (columns a-d, f, g), did not give significant fluorescence. We also tested a known protein persulfidation method using H₂O₂ and H₂S. When the concentrations of

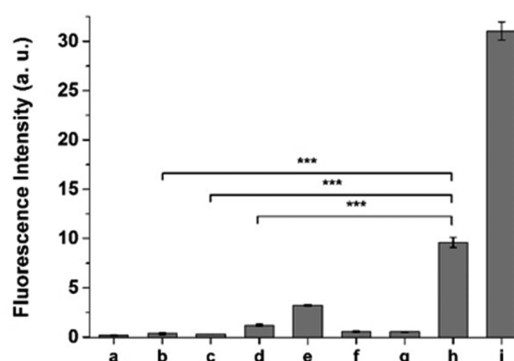


Figure 7. Thioglucose-GOx induced BSA S-persulfidation detected by SSP4. In these studies, 30 μ M reduced BSA and 10 μ M SSP4 were used. Fluorescence response was recorded at 515 nm. (a) SSP4 only, (b) SSP4 + BSA, (c) SSP4 + BSA + Na₂S (60 μ M), (d) SSP4 + BSA + H₂O₂ (60 μ M) + CAT (25 μ g/mL), (e) SSP4 + BSA + Na₂S (60 μ M) + H₂O₂ (60 μ M) + CAT (25 μ g/mL) (f) SSP4 + BSA + thioglucose (200 μ M), (g) SSP4 + BSA + GOx (10 μ g/mL), (h) SSP4 + BSA + thioglucose (200 μ M) + GOx (10 μ g/mL) + CAT (25 μ g/mL), (i) BSA (600 μ M) + H₂O₂ (4 mM) + CAT (25 μ g/mL) + Na₂S (2 mM) + SSP4. The experiments were performed in triplicate, and results are expressed as mean \pm SD ($n = 3$). Statistical analysis was performed using one-way ANOVA. *** $P < 0.001$.

H₂O₂ and H₂S were similar to those of thioglucose-GOx system (e.g., 60 μ M H₂O₂ and 60 μ M Na₂S, shown in column e), we only observe weak fluorescence. When much higher concentrations of the reagents were used (600 μ M BSA with 4 mM H₂O₂ and 2 mM Na₂S) we were able to observe strong fluorescent signals (column i). It should be noted that these high concentrations are unrealistic for real biological applications. Therefore, our results indicated that thioglucose-GOx is an efficient method to cause protein persulfidation under physiologically relevant H₂O₂/H₂S concentrations and SSP4 is suitable for the detection of protein S-persulfidation.

The formation of BSA persulfide was further confirmed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Briefly, BSA was treated with thioglucose-GOx as described above. The resulted protein was then incubated with β -(4-hydroxyphenyl)ethyl iodoacetamide (HPE-IAM, 10 mM) to block the -SH and -SSH. Controls using untreated BSA were also performed. Proteins were then digested with trypsin and subjected to LC-MS/MS. As shown in Figure 8, in thioglucose/GOx treated samples the extracted ion chromatogram (XIC) signals clearly showed a substantial level of persulfide (-SSH) adduct on peptide GLVLIAFSQYLQQCPF-DEHVK (Figure 8C). The site of persulfidation (Cys34) was confirmed by higher-energy collision dissociation (HCD) MS/MS (Figure 8D). Instead, the main observed peptide species containing Cys34 in untreated BSA was the Cys34-thiol (-SH) alkylated form (Figure 8A/B). In addition to BSA we also tested this thioglucose/GOx method with two other proteins-papain and GAPDH. Effective persulfidation was obtained on both proteins (Figures S8 and S9 in Supporting Information).

It was interesting to discover that the slow and steady production of H₂S and H₂O₂ from thioglucose/GOx acted as a more efficient protein persulfidation system than the direct addition of H₂S and H₂O₂, even at similar concentrations. While the detailed mechanism is still unclear, we propose the following explanations (Scheme 2): In previous studies, protein persulfidation was normally achieved by sequential treatments with high concentrations of H₂O₂ and then H₂S. A

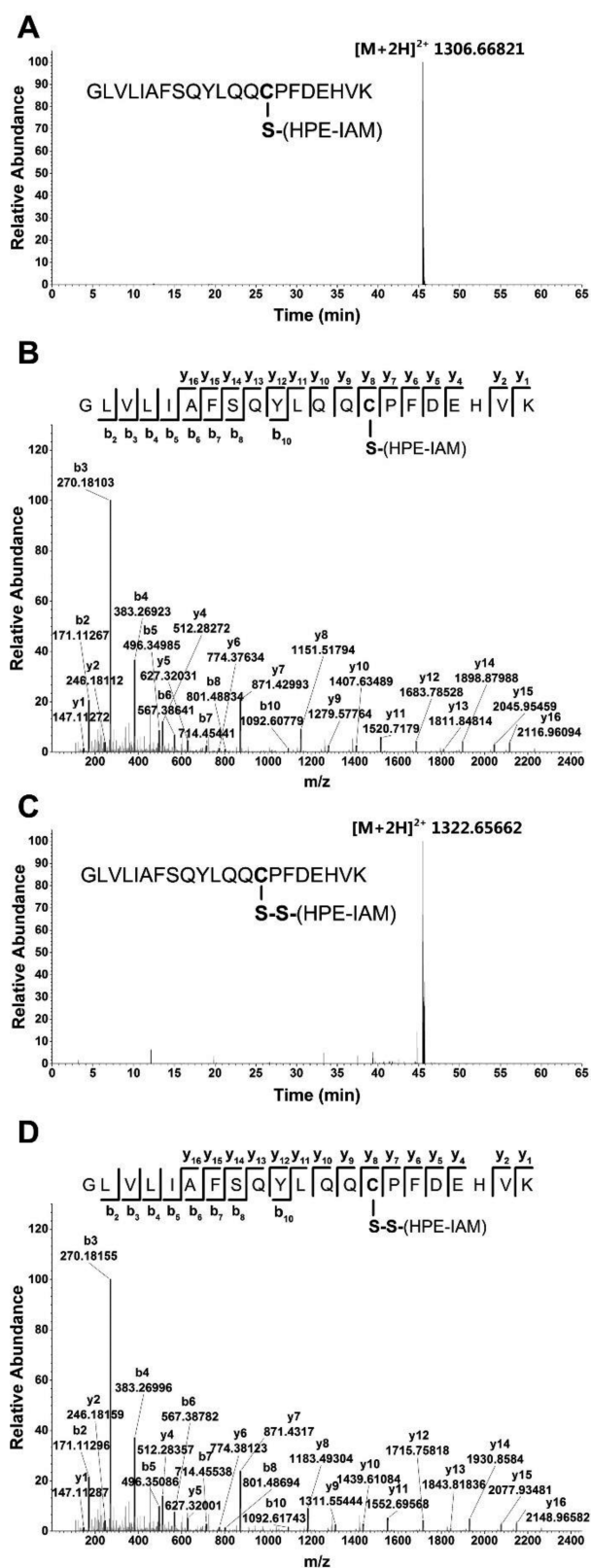
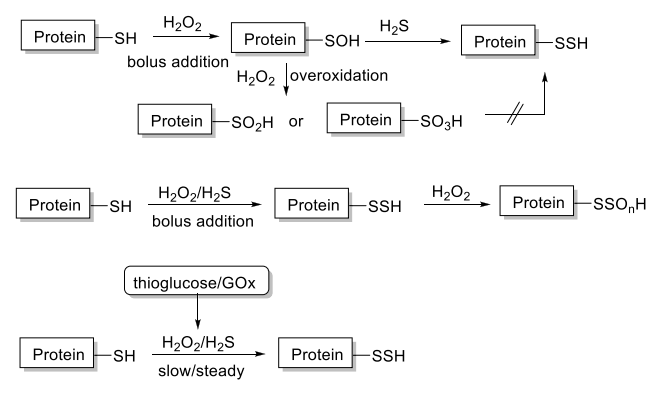


Figure 8. Extracted ion chromatograms (XIC) and MS/MS spectra of the HPE-IAM labeled Cys34 containing peptide (GLVLIAFSQYLQCCPFDEHVK) from BSA, with or without the treatment of thioglucose/GOx. (A) XIC of the $-SH$ peptide, (B) MS/MS spectrum of the $-SH$ peptide, (C) XIC of the $-SSH$ peptide, (D) MS/MS spectrum of the $-SSH$ peptide.

Scheme 2. Proposed Explanations on High Efficiency of Protein Persulfidation by the Thioglucose/GOx System



problem with this method is overoxidation in the first step, which produces protein sulfenic acid ($P-SO_2H$) or sulfonic acid ($P-SO_3H$). These species cannot be converted to $P-SSH$ by H_2S . This overoxidation may exist when BSA is treated with bolus additions of H_2O_2/H_2S pair, which diminishes the efficiency of persulfidation. Another possibility is that excess H_2O_2 would always exist in bolus additions and it could rapidly react with the newly formed protein persulfides and therefore, decrease persulfidation. It is known that persulfides ($RSSH$) are highly reactive to H_2O_2 to form $RSSO_nH$.^{34,35} On the other hand, the slow and steady H_2O_2/H_2S production from thioglucose/GOx may be able to avoid the presence of excess H_2O_2 in the system, thus preventing the overoxidation reactions.

It was also interesting to see that thioglucose/GOx induced persulfidation worked effectively on proteins but not on small molecules. This could be attributed to two reasons: (1) H_2O_2 -oxidation works less effectively on small molecule thiols as compared to proteins. We analyzed the total $-SH$ contents in the reaction between Cys and thioglucose/GOx. As shown in Figure S7, only a minor thiol concentration decrease in this reaction was observed, suggesting Cys oxidation in this system was slow. (2) Even small molecule thiols react with H_2O_2 ; the resultant sulfenic acids ($RSOH$) are much more unstable than protein-derived sulfenic acids. They should further react with another molecule of thiol to form a disulfide. As such, persulfide formation on small molecules in this system will not be feasible.

Having demonstrated that thioglucose-GOx is a controllable platform for H_2S/H_2O_2 dual release under biologically friendly environments, we wondered if we could further tune the releasability with a dual enzyme system or multistimuli response. In this strategy, thioglucose could be considered as a caged H_2S/H_2O_2 vehicle. Engineering this motif with another triggering group would enable their generation in response to specific biological stimuli. To test this hypothesis, we prepared 1-thio- β -D-lactose (thiolactose) which could be hydrolyzed by β -galactosidase (β -Gal) to form galactose and thioglucose. As such, only under the dual-enzyme catalysis (β -Gal and GOx) will it produce H_2S and H_2O_2 . As shown in Figure 9, thiolactose was stable in PBS buffers. It did not release H_2S under the treatment of either β -Gal or GOx. However, in the presence of both β -Gal and GOx, a time-dependent H_2S formation was observed with the peak concentration of $\sim 16 \mu M$ in about 2 h. The capability of H_2O_2 delivery from this thiolactose-dual enzyme system was also confirmed by AP/

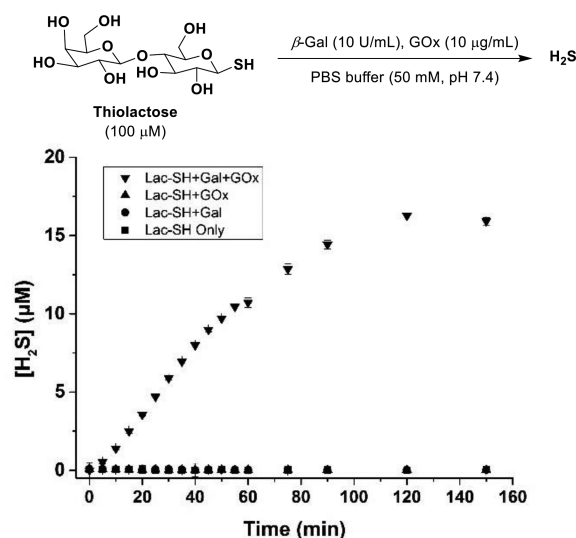


Figure 9. H₂S release profile of thiolactose (100 μM) in the presence of β-Gal (10 U/mL) and GOx (10 μg/mL) in PBS buffer (50 mM, pH 7.4). The experiments were performed in triplicate, and results are expressed as mean ± SD (*n* = 3).

HRP assay under the same conditions (Figure S10). It is worth noting that elevated lysosomal β-Gal activity has been well-known as an important biomarker for senescent cells and primary ovarian cancers.^{36,37} Also H₂S exhibits protective effects against cellular senescence and certain cancers.^{38,39} Thus, β-Gal activated H₂S donors like thiolactose might be explored as potential antiaging or anticancer agents.

To further demonstrate the tunability of the thioglucose-GOx platform, we next synthesized thioglucose disulfide. This symmetrical glucosyl disulfide is expected to undergo disulfide-bond cleavage induced by biothiols to form thioglucose, which could subsequently release H₂S and H₂O₂ in the presence of GOx. To test this idea, thioglucose disulfide (50 μM) was incubated with GSH (0–1000 μM) in PBS (50 mM, pH 7.4) containing GOx (10 μg/mL), and its H₂S production was monitored by MB assay. As shown in Figure 10, thioglucose disulfide did not produce detectable H₂S in the absence of GSH. However, in the presence of GSH, thioglucose disulfide exhibited dose- and time-dependent H₂S-releasing. The formation of H₂O₂ was also confirmed using AP/HRP assay (Figure S11). These results indicate that thioglucose disulfide is an efficient thiol-activated H₂S/H₂O₂ donor catalyzed by GOx.

CONCLUSIONS

Compounds that can release redox regulating molecules such as H₂S and H₂O₂ are not only useful research tools but also potential therapeutic agents. While many such compounds have been reported, a common problem is that they also produce organic byproducts that could cause unwanted side effects. This problem should be considered in the development of next generation donor templates. In addition, current efforts in this field tend to focus on individual regulating species, which could miss the important crosstalk and synergistic effects between multiple species. Nevertheless, the coexistence of these species is real in nature but donors that can release multiple ROS/RSS are still unavailable. In this work, we attempted to solve the aforementioned challenges by developing novel dual-releasing donor templates. We have

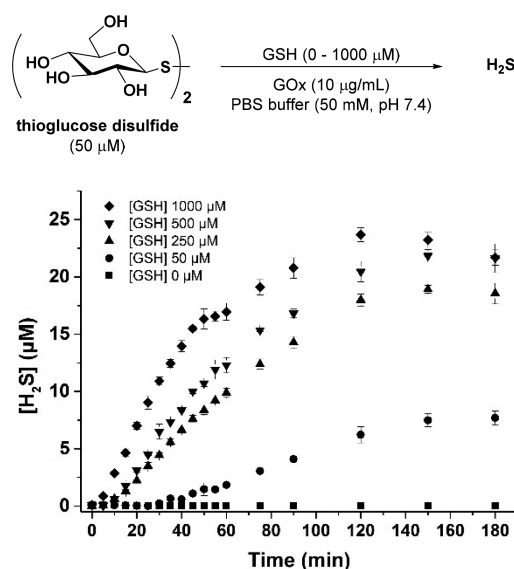


Figure 10. H₂S release profile of thioglucose disulfide (50 μM) in the presence of GSH (0 μM, 50 μM, 250 μM, 500 μM and 1000 μM) and GOx (10 μg/mL) in PBS buffer (50 mM, pH 7.4). The experiments were performed in triplicate, and results are expressed as mean ± SD (*n* = 3).

demonstrated that thioglucose combined with GOx can serve as a new platform for controlled H₂S/H₂O₂ dual release with no bio-unfriendly byproducts. The generation of H₂O₂ in this system did not affect H₂S release. This thioglucose-GOx system was used to effectively induce protein S-persulfidation. Moreover, thioglucose is a highly tunable motif and can be readily modified to introduce additional release-control factors so other biologically relevant stimuli can be used to regulate the delivery of H₂S/H₂O₂. We expect this thiosugar-GOx platform will be a useful tool for elucidating the mechanisms of H₂S/H₂O₂ signaling and promoting H₂S based therapeutic applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.1c06372>.

Compound characterizations, experimental protocols, additional experimental data (PDF)

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Notes

The authors declare no competing financial interest.

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