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A Sweet H_2S/H_2O_2 Dual Release System and Specific Protein S-Persulfidation Mediated by Thioglucose/Glucose Oxidase

Xiang Ni, Xiaolu Li, Tun-Li Shen, Wei-Jun Qian, and Ming Xian*

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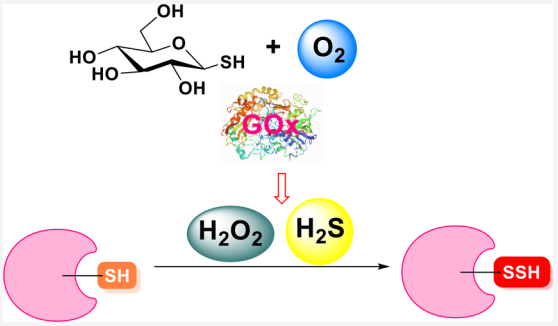
ABSTRACT: H_2S and H_2O_2 are two redox regulating molecules that play important roles in many physiological and pathological processes. While each of them has distinct biosynthetic pathways and signaling mechanisms, the crosstalk between these two species is also known to cause critical biological responses such as protein S-persulfidation. So far, many chemical tools for the studies of H_2S and H_2O_2 have been developed, such as the donors and sensors for H_2S and H_2O_2. However, these tools are normally targeting single species (e.g., only H_2S or only H_2O_2). As such, the crosstalk and synergetic effects between H_2S and H_2O_2 have hardly been studied with those tools. In this work, we report a unique H_2S/H_2O_2 dual donor system by employing 1-thio-β-D-glucose and glucose oxidase (GOx) as the substrates. This enzymatic system can simultaneously produce H_2S and H_2O_2 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 H_2S/H_2O_2. This dual release system should be useful for future research on H_2S and H_2O_2.



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, H_2S is endogenously produced by enzymes including cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and 3-mercaptopyruvate sulfur transferase (3-MST). Dysregulated H_2S levels are associated with pathological processes like cancer, inflammation, hypertension, etc.[1,2] As the center species of ROS, H_2O_2 is formed from spontaneous dismutation of superoxide ($O_2^{\cdot-}$) or under the catalysis of superoxide dismutase (SOD). Endogenous H_2O_2 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 H_2O_2 leads to oxidative stress and damage, which is connected to aging and neurodegenerative diseases.[5,6] While H_2S and H_2O_2 have their very distinct signaling pathways, they also interact with each other and work collectively in redox signaling.[7] The direct reaction between H_2S and H_2O_2 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 H_2S. However, H_2S 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 H_2S and sulfenic acid (S−OH), which is the oxidation product from H_2O_2.[9] As such, an appropriate level of H_2O_2 is required to facilitate H_2S signaling cascades, especially under oxidative stress. In another study H_2S was found to significantly amplify H_2O_2-based therapeutic treatment for cancer in a mouse model.[10] Moreover, the mechanism of acute H_2S intoxication is due to H_2S 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 H_2S and H_2O_2 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 H_2S donors have been reported and they can be triggered by different cellular factors to release H_2S.[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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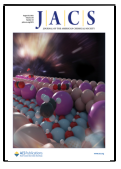

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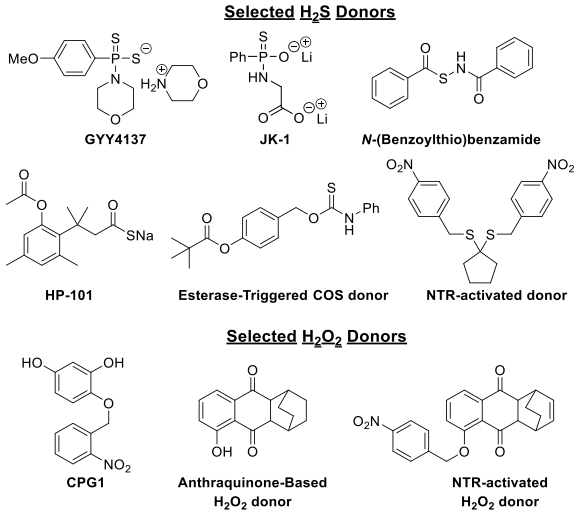


Figure 1. Representative examples of synthetic H_2S and H_2O_2 donors.

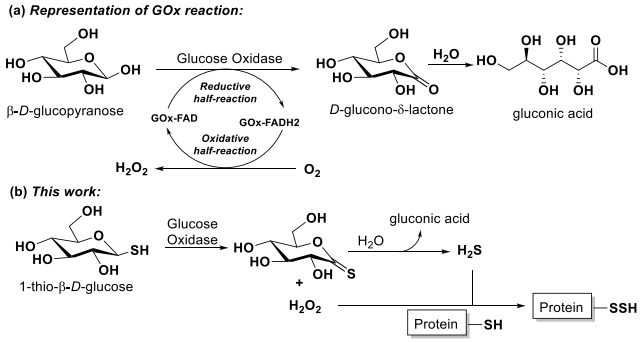
anhydrase (CA)-triggered COS/H_2S donors, and nitroreductase-activated donors.[15−17] H_2O_2 donors are much less developed, and researchers still tend to use H_2O_2 directly in studies. However, it has been demonstrated that bolus delivery of H_2O_2 is often problematic due to rapid consumption of H_2O_2 in biological systems such as in cells.[18,19] This justifies the need for slow and continuous H_2O_2 releasing methods. Currently only a few hydroquinone and anthraquinone derivatives are reported as H_2O_2 donors (Figure 1).[20−22]

While these donor compounds have advanced our understanding of H_2S and H_2O_2, limitations still remain: (1) All these donors are synthetic materials. Inevitably, they will also produce significant amounts of organic byproducts (in addition to H_2S or H_2O_2). This could cause unwanted side effects. (2) Enzyme-triggered donors (especially H_2O_2 donors) are still very limited. (3) All available donors can only produce one species (H_2S or H_2O_2), which can hardly mimic the concurrent presence of H_2S and H_2O_2 or allow the study of their crosstalk and synergetic effects. Herein, we would like to report a new H_2S/H_2O_2 dual donor system employing thioglucose and GOx. This can produce H_2S and H_2O_2 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 H_2S/H_2O_2 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 O_2 gas with the production of H_2O_2 (Scheme 1a).[23] Because of this property, the glucose/GOx system has been used as an alternative for the exogenous addition of H_2O_2 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 H_2S/H_2O_2 and Induce Protein S-Persulfidation



which should easily undergo hydrolysis to release H_2S. Therefore, we envisioned that thioglucose/GOx could be a unique enzyme triggered H_2S/H_2O_2 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 H_2S/H_2O_2 might be an efficient way to induce protein S-persulfidation. Furthermore, the thioglucose moiety can be engineered as stimuli-response prodrugs to deliver H_2S and H_2O_2 upon specific biologically relevant activation.

With this idea in mind, we first evaluated H_2S release from thioglucose in the presence of GOx. The standard methylene blue (MB) method was used to quantify H_2S production. As shown in Figure 2, thioglucose itself was found to be stable in

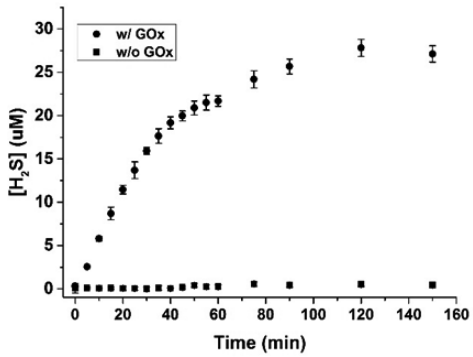


Figure 2. H_2S release profile of thioglucose (100 μM) in the presence and absence of GOx (10 μg/mL) in PBS buffer (50 mM, pH 7.4). The experiments were performed in triplicate, and results are expressed as mean ± SD (n = 3).

buffers and no obvious H_2S release was detected in the absence of GOx. However, significant H_2S 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 H_2S generation. The concentration of H_2S reached the maximum value of ~28 μM in about 2 h and then slowly decreased presumably due to volatilization of H_2S gas.

The H_2S formation was further verified by fluorescence measurements with the H_2S-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)

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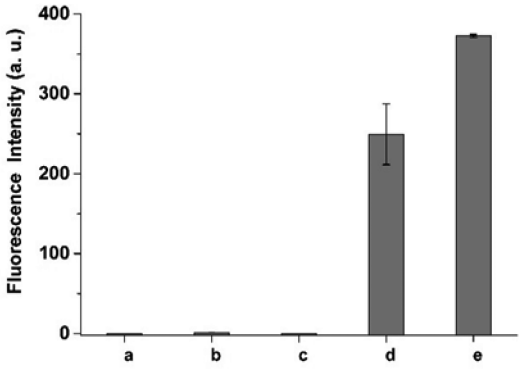

Figure 3. H_2S release from thioglucose (100 μM) in the presence of GOx (10 μg/mL) detected by WSP5 (10 μM) in PBS buffer (50 mM, pH 7.4). (a) WSP5 only, (b) WSP5 + thioglucose, (c) WSP5 + GOx, (d) WSP5 + thioglucose + GOx, (e) WSP5 + 100 μM Na_2S. The experiments were performed in triplicate, and results are expressed as mean ± SD (n = 3).

and GOx (10 μg/mL) at room temperature for 1 h, indicating that thioglucose indeed produced H_2S in the presence of GOx.

We next monitored the formation of H_2O_2 by the ferrous oxidation−xylenol orange method (FOX 1 assay) under the optimized conditions described above. As shown in Figure 4, a

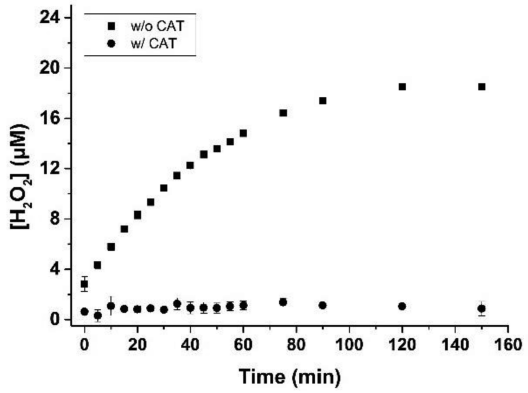

Figure 4. Time-dependent generation of H_2O_2 from thioglucose (100 μM) and GOx (10 μg/mL) in PBS buffer (50 mM, pH 7.4) in the presence and absence of CAT (50 μg/mL). The experiments were performed in triplicate, and results are expressed as mean ± SD (n = 3).

time-dependent H_2O_2 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 H_2O_2 was completely scavenged. The H_2O_2 formation was also determined by fluorescence measurements with H_2O_2-sensitive Amplex Red/horseradish peroxidase (AP/HRP) assay (Figure S5). Interestingly, we noticed that the release of H_2S was not affected by the addition of CAT (Figure S2), indicating that thioglucose could be used as a clean H_2S donor if two enzymes (GOx and CAT) were applied.

The concurrent formation of H_2S and H_2O_2 in this system could lead to a concern that H_2S and H_2O_2 might react with each other, so the release of H_2S 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 H_2S and H_2O_2 is 0.73 M^{-1} s^{-1} (pH 7.4, 37 °C), with the formation of a mixture of hydrogen polysulfide (H_2S_n) as the possible products.[27] We also wondered if this system could produce H_2S_n. To test this, a specific fluorescent probe PSP-3[28] was used to monitor the generation of H_2S_n. As shown in Figure 5, negligible

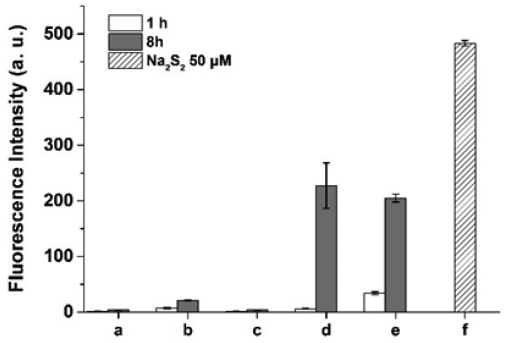

Figure 5. Detection of H_2S_n produced from thioglucose-GOx by PSP-3 (10 μM). Thioglucose (100 μM) was incubated with GOx (10 μg/mL) and PSP-3 in PBS buffer (50 mM, pH 7.4) for 1 h (white) and 8 h (gray), respectively. Fluorescence responses were recorded at 515 nm. (a) PSP-3 only, (b) PSP-3 + thioglucose, (c) PSP-3 + GOx, (d) PSP-3 + thioglucose + GOx, (e) PSP-3 + Na_2S (100 μM) + H_2O_2 (100 μM), (f) PSP-3 + Na_2S_2 (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 H_2O_2 and Na_2S. Weak fluorescence signals were observed with the incubation of a mixture of Na_2S (100 μM) and H_2O_2 (100 μM) after 1 h while much stronger fluorescence was noted after 8 h. These results again suggested that H_2S and H_2O_2 could react to form H_2S_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 H_2S and H_2O_2 under biologically relevant concentrations. We next wondered if this system could be used to induce protein S-persulfidation, an important posttranslational modification mediated by H_2S.[9,29] This hypothesis was based on the knowledge that H_2S alone can hardly induce protein S-persulfidation (as the reaction between H_2S and protein disulfides is usually slow and less-productive). H_2O_2 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 H_2S. However, this method needs very high concentrations of H_2O_2 (4 mM) and H_2S (2 mM).[31] We expected that our slow and continuous H_2S/H_2O_2 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

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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

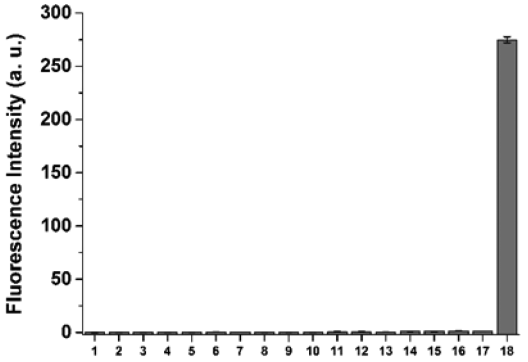

Figure 6. Fluorescence responses of SSP4 toward various low molecular weight biothiols in the presence of thioglucose-GOx. SSP4 (10 μM) was incubated with different substrates in PBS buffer (50 mM, pH 7.4) for 1 h, then the fluorescence responses were recorded at 515 nm. (1) SSP4 only, (2) SSP4 + thioglucose (100 μM), (3) SSP4 + GOx (10 μg/mL), (4) SSP4 + Cys (100 μM), (5) SSP4 + GSH (1 mM), (6) SSP4 + thioglucose (100 μM) + Cys(100 μM), (7) SSP4 + thioglucose (100 μM) + GSH (1 mM), (8) SSP4 + GOx (10 μg/mL) + Cys(100 μM), (9) SSP4 + GOx (10 μg/mL) + GSH(1 mM), (10) SSP4+ thioglucose (100 μM) + GOx (10 μg/mL), (11) SSP4 + Na_2S (30 μM) + H_2O_2 (20 μM), (12) SSP4+ thioglucose (100 μM) + GOx (10 μg/mL) + Cys(100 μM), (13) SSP4+ thioglucose (100 μM) + GOx (10 μg/mL) + GSH(1 mM), (14) SSP4+ thioglucose (100 μM) + Na_2S (30 μM) + H_2O_2 (20 μM), (15) SSP4 + GOx (10 μg/mL)+ Na_2S (30 μM) + H_2O_2 (20 μM), (16) SSP4+ Cys(100 μM) + Na_2S (30 μM) + H_2O_2 (20 μM), (17) SSP4 + GSH (1 mM) + Na_2S (30 μM) + H_2O_2 (20 μM), (18) SSP4 + Na_2S_2 (50 μM). The experiments were performed in triplicate, and results are expressed as mean ± 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 Na_2S/H_2O_2 under similar concentrations of the enzyme-promoted system (column 11). Furthermore, exposure of biothiols to thioglucose-GOx or the corresponding amounts of Na_2S/H_2O_2 showed no fluorescence increase (columns 12−17). As a positive control, SSP4 showed high fluorescence response to Na_2S_2 (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 μM) was incubated with thioglucose (200 μM) and GOx (10 μg/mL) in PBS at room temperature for 1 h. CAT (25 μg/mL) was then added to remove excess H_2O_2. SSP4 (10 μM) 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 H_2O_2 and H_2S. When the concentrations of

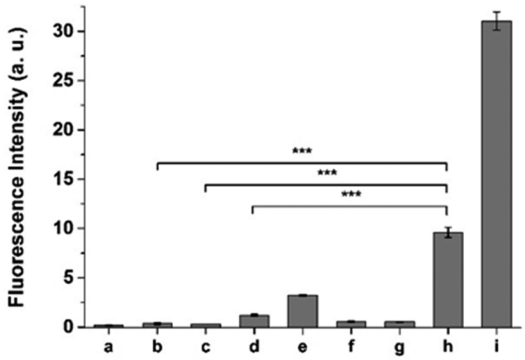

Figure 7. Thioglucose-GOx induced BSA S-persulfidation detected by SSP4. In these studies, 30 μM reduced BSA and 10 μM SSP4 were used. Fluorescence response was recorded at 515 nm. (a) SSP4 only, (b) SSP4 + BSA, (c) SSP4 + BSA + Na_2S (60 μM), (d) SSP4 + BSA + H_2O_2 (60 μM) + CAT (25 μg/mL), (e) SSP4 + BSA + Na_2S (60 μM) + H_2O_2 (60 μM) + CAT (25 μg/mL) (f) SSP4 + BSA + thioglucose (200 μM), (g) SSP4 + BSA + GOx (10 μg/mL), (h) SSP4 + BSA + thioglucose (200 μM) + GOx (10 μg/mL) + CAT (25 μg/mL), (i) BSA (600 μM) + H_2O_2 (4 mM) + CAT (25 μg/mL) + Na_2S (2 mM) + SSP4. The experiments were performed in triplicate, and results are expressed as mean ± SD (n = 3). Statistical analysis was performed using one-way ANOVA. ***P < 0.001.

H_2O_2 and H_2S were similar to those of thioglucose-GOx system (e.g., 60 μM H_2O_2 and 60 μM Na_2S, shown in column e), we only observe weak fluorescence. When much higher concentrations of the reagents were used (600 μM BSA with 4 mM H_2O_2 and 2 mM Na_2S) 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 H_2O_2/H_2S 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 H_2S and H_2O_2 from thioglucose/GOx acted as a more efficient protein persulfidation system than the direct addition of H_2S and H_2O_2, 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 H_2O_2 and then H_2S. A

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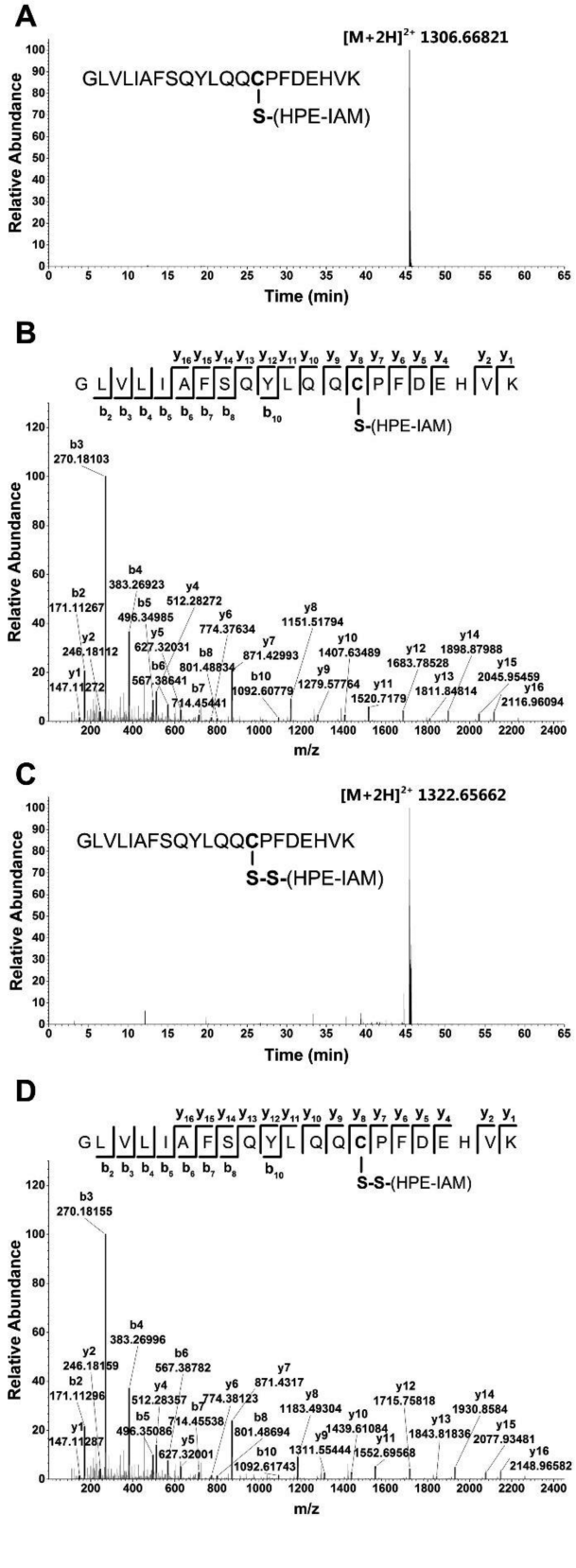
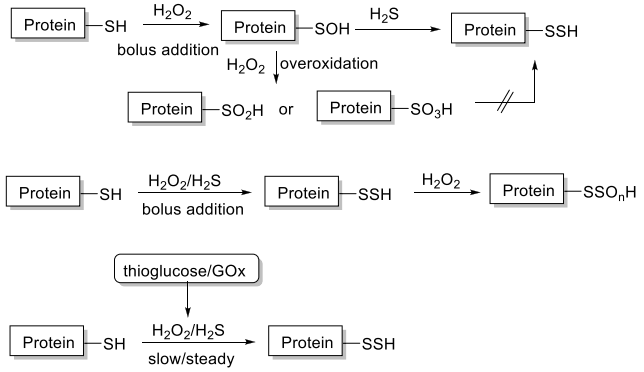


Figure 8. Extracted ion chromatograms (XIC) and MS/MS spectra of the HPE-IAM labeled Cys34 containing peptide (GLVLIAFSQYLQQCPFDEHVK) from BSA, with or without the treatment of thioglucose/GOx. (A) XIC of the −SH peptide, (B) MS/MS spectrum of the −SH peptide, (C) XIC of the −SSH peptide, (D) MS/MS spectrum 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 sulfinic 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 ∼16 μM in about 2 h. The capability of H_2O_2 delivery from this thiolactose-dual enzyme system was also confirmed by AP/

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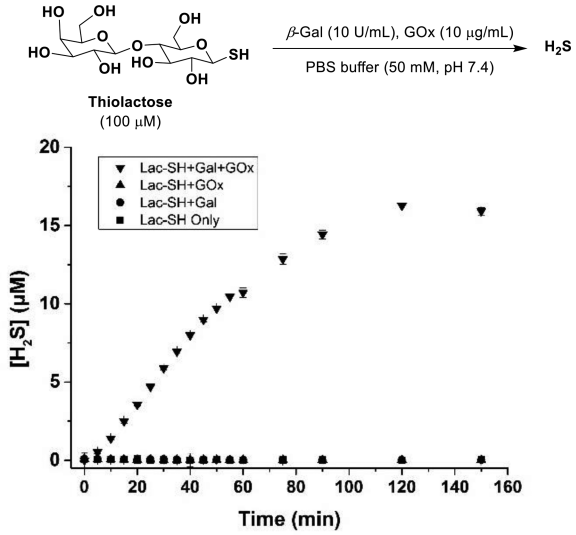

Figure 9. H_2S release profile of thiolactose (100 μM) in the presence of β-Gal (10 U/mL) and GOx (10 μg/mL) in PBS buffer (50 mM, pH 7.4). The experiments were performed in triplicate, and results are expressed as mean ± SD (n = 3).

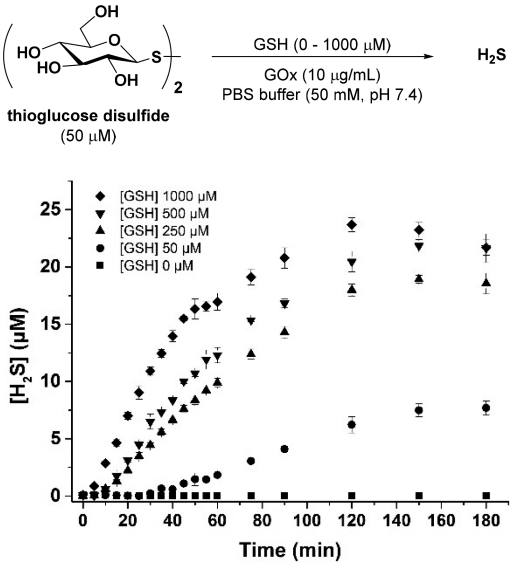

Figure 10. H_2S release profile of thioglucose disulfide (50 μM) in the presence of GSH (0 μM, 50 μM, 250 μM, 500 μM and 1000 μM) and GOx (10 μg/mL) in PBS buffer (50 mM, pH 7.4). The experiments were performed in triplicate, and results are expressed as mean ± SD (n = 3).

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 H_2S exhibits protective effects against cellular senescence and certain cancers.[38,39] Thus, β-Gal activated H_2S 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 H_2S and H_2O_2 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 H_2S production was monitored by MB assay. As shown in Figure 10, thioglucose disulfide did not produce detectable H_2S in the absence of GSH. However, in the presence of GSH, thioglucose disulfide exhibited dose- and time-dependent H_2S-releasing. The formation of H_2O_2 was also confirmed using AP/HRP assay (Figure S11). These results indicate that thioglucose disulfide is an efficient thiol-activated H_2S/H_2O_2 donor catalyzed by GOx.

CONCLUSIONS

Compounds that can release redox regulating molecules such as H_2S and H_2O_2 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 demonstrated that thioglucose combined with GOx can serve as a new platform for controlled H_2S/H_2O_2 dual release with no bio-unfriendly byproducts. The generation of H_2O_2 in this system did not affect H_2S 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 H_2S/H_2O_2. We expect this thiosugar-GOx platform will be a useful tool for elucidating the mechanisms of H_2S/H_2O_2 signaling and promoting H_2S 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)

AUTHOR INFORMATION

Corresponding Author

Ming Xian − *Department of Chemistry, Brown University, Providence, Rhode Island 02912, United States;* orcid.org/0000-0002-7902-2987; Email: ming_xian@brown.edu

Authors

Xiang Ni − *Department of Chemistry, Brown University, Providence, Rhode Island 02912, United States*

Xiaolu Li − *Biological Sciences Division, Pacific Northwest National Laboratory, Richland, Washington 99352, United States*

Tun-Li Shen − *Department of Chemistry, Brown University, Providence, Rhode Island 02912, United States*

Wei-Jun Qian − *Biological Sciences Division, Pacific Northwest National Laboratory, Richland, Washington 99352, United States;* orcid.org/0000-0002-5393-2827

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.1c06372

Notes

The authors declare no competing financial interest.

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